

ANNUAL REVIEW OF PHYSIOLOGY



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# ANNUAL REVIEW OF PHYSIOLOGY

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## PREFACE

The *Annual Review of Physiology* was founded to serve a unique function—that of making available to the scientific world a survey at yearly periods of significant developments in physiology which came from the efforts of workers in all nations. Although published in the United States it was to be international in scope—in authorship, coverage of literature, and in distribution. In spite of the inauspicious date of our first volume—1939—the Editorial Committee has striven and with some success to maintain the international character of the *Review* during the war years.

We are now happy to be able to include in the present volume contributions from Great Britain, New Zealand, Belgium, Denmark, and Switzerland. It is with particular satisfaction that we have secured for our next volume the promise of two reviews from among our colleagues in Russia, hitherto unrepresented in the *Annual Review of Physiology*.

Other evidences of the returning unity of the scientific world are the restoration of delivery of the *Annual Review* to subscribers in countries occupied by the enemy during the war, and the reappearance in large number of citations of physiological advances made by workers in these countries. We take this occasion "to pay tribute to our colleagues from Europe, who exhibited remarkable fortitude during the German occupation in carrying on their scientific work under difficult conditions, and often in addition to their activities as leaders or participants in the underground resistance."<sup>1</sup>

A few reports of work done in enemy countries will be found inconspicuously in their proper places, one such report receives extended consideration. It is our declared policy that the inclusion or exclusion of material and the emphasis to be placed on it shall be decided solely on the extent to which that particular work has been pursued in accordance with the requirements of the scientific method, and not on the political, economic, or social conditions of their countries, or on the views held by the workers themselves. To the extent that scientists of the countries with which we have been at war conform to these requirements, we welcome them back into the international scientific fold.

<sup>1</sup> Appropriated for the Preface from the review of Grundfest in this volume.

The bulk of physiological material deserving review, always unwieldy, has been considerably augmented by the publication of research, carried out during the war but kept from the light by entanglement in "security" regulations or by being buried under the pressure of other duties. The release of this material has made it even more difficult for our reviewers to do critical justice to the year's output within the confines of their allotted space. Many contributors apparently found it impossible and, sending us manuscripts up to twice the assigned length, forced the editorial staff to make extensive excisions, often of valuable material. For the necessity of doing this, we extend our apologies to those of our reviewers whose manuscripts have suffered.

Every year one or more reviews promised in our announcements fail, through various turns of fortune, to reach the final stage of preparation. Our casualty list this year includes the review on Special Senses by Dr. A. Miles, a victim of illness, and that on Shock by Drs. C. N. H. Long and A. E. Wilhelmi, deferred to our next volume.

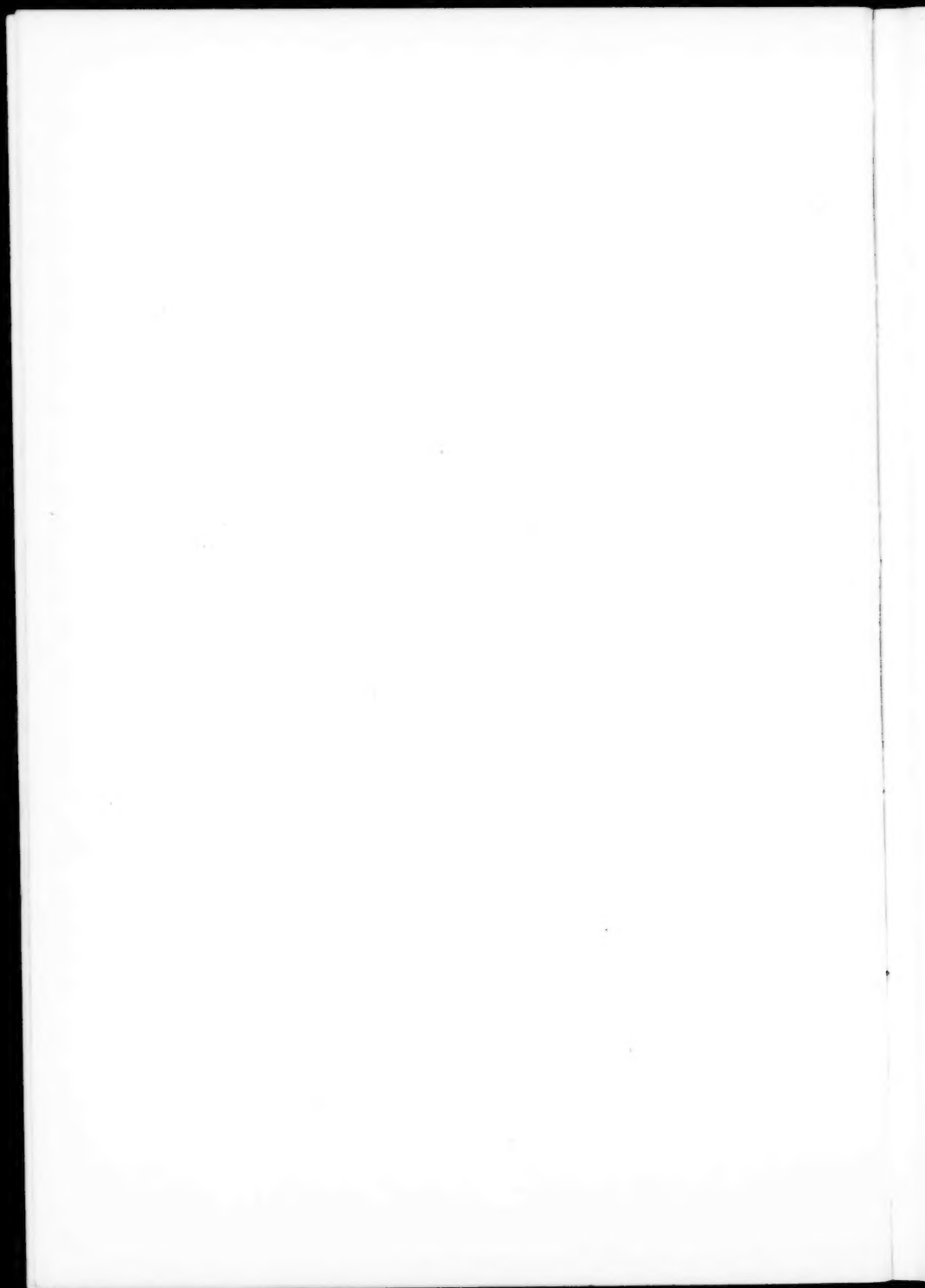
The present volume marks the inauguration of a new editorial staff. With the addition of the *Annual Review of Microbiology* to the family of Annual Reviews, Inc., Professor James Murray Luck, pressed by the multiplication of duties which even his manifold powers could not sustain, has relinquished the editorship of the *Annual Review of Physiology*. Lest there be any fear of the consequences, we hasten to add that behind the scenes he still exercises general editorial supervision over all three of the *Reviews*. Although an envoi is accordingly not appropriate, the Editorial Committee and staff seize the occasion to acknowledge Dr. Luck's services, as founder, editor, and business manager to which a considerable measure of the success of the *Review* has been due.

We wish to thank our editorial and secretarial assistants, Barbara Davey, Virginia Silveira, Ruth Swan, Margaret Galloway, Jane Gerhart, and Geraldine Fuhrman for their indispensable contributions, and finally, to note that the George Banta Publishing Company continues to delight us with the efficient service which has done so much to smooth the editorial path.

A.J.C.	M.H.J.
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## GROWTH<sup>1</sup>

BY STANLEY P. REIMANN

*The Lankenau Hospital Research Institute and The Institute for  
Cancer Research, Philadelphia Pennsylvania*

Since only a limited field can be discussed in a review such as this, the decision has been to emphasize pathological aspects of growth. The studies on the pathology of growth have not always been well correlated with the general trends of biological thought, nor have they always kept pace with the knowledge and concepts of normal growth as the latter have developed during the last few years. It is helpful to compare the normal with the pathological and vice versa, since the pathological often has given clues and helped to clarify the normal. In pathology, it will be remembered, not only are tumors considered among growth problems, reviews of which are appearing from time to time (1, 2, 3), but such fields as hyperplasias, hypoplasias, replacement fibrosis, wound healing, regeneration, and others fall in this category, not to mention the growth factors of bacteria, protozoa, metazoan parasites, and the effects of nutritional substances. To classify and catalogue adequately the various aspects of growth, a frame of reference is required into which new and old facts, as well as other related knowledge can be conveniently fitted. Two such frames of reference have been formulated, one by Weiss (4) and another by Hammett (5) [see also (6, 7, 8)].

### INDUCTION OR EVOCATION OF ORGANIZATION

The subject of inductors or evocators or organizers (or several other terms) continues to occupy the attention of a number of biologists, as for example, Holtfreter (9, 10). Reviews have appeared recently (11) and information is presented on the chemical aspects of induction. Important as are the findings and the concepts which they have "evocated," it is a fact that not much attention has been devoted to them by pathologists. Therefore, an article by Thoma and Goldman (12) is singled out for comment, especially the paragraphs (pp. 445-46) in which speculations on

<sup>1</sup> This review covers the period from 1940 to 1946.



inductive effects appear. In this paper odontogenic tumors are classified as epithelial, mesenchymal, and mixed.

In tumors of simple structure, such as carcinomas, in which only one variety of cell is neoplastic, the stroma grows along (induced by the tumor cells?) functioning as support for the tumor cells and for the necessary vessels. When the tumor cells are destroyed, as can be done by irradiation, leaving the stroma cells relatively uninjured, as observed anatomically and clinically, the latter do not continue to grow autonomously to produce a sarcoma but shrink to a scar or disappear. (An alternative explanation is that the irradiation has destroyed them also and that new connective tissue grows in to produce the eventual scar.) But in the case of mixed tumors (odontogenic, salivary gland, and others) the connective tissue cells are also autonomous. This is seen clearly in malignant mixed tumors in which the epithelium may metastasize at one time and connective tissue at another. In such tumors induction may occur (12). But which tissue induces the other, or whether the induction is mutual from the beginning of the tumor is not known. Even if induction operates at the start of such tumors, both very shortly become autonomous, if either was not so in the beginning. As stated, this is quite different from the stroma in ordinary carcinomas and also somewhat different from the "benign" (nonmetastasizing) mixed tumors such as are 90 per cent or more of salivary gland tumors (13, 14).

As an alternative consideration, it is possible that in the case of either benign or malignant mixed tumors the cells of epithelial and connective tissue are both descendants of the cell or cells which originally gave rise to the tumor. If this be so, induction on the part of one or the other type of tumor cell has nothing to do with the case. Some other factor determines the potency. These speculations lead to the whole subject of potency, determination, fields, and organizers.

The potency of an undifferentiated or partially differentiated cell is greater than its developmental fate (15). Is this true also of tumor cells? Malignant tumor cells once established do not seem to respond to normal inductors, organizers or fields, insofar as altering their differentiations and organizing responses to the organism as a whole are concerned, as, for example, shown by the findings of Briggs & Grant (16). They transplanted bits of frog tumor into various sites in young tadpoles known to be capable of regen-

eration and therefore presumably possessing morphogenetic "fields." The implanted tumors grew and retained their characteristic morphology indicating that the "fields," if operating under the conditions of the experiment, had no effect on the tumor.

We cannot enter here into further discussion of a "new race of cells," "somatic mutation," etc. (17). A hypothesis, presented by Jack Schultz at the 1946 Gibson Island Conference, enables him to integrate a large mass of data coming from various fields. As cells differentiate the constitution of their nuclei changes along with their cytoplasm, i.e., the nuclei and cytoplasm are interdependent in differentiation. The nature of nuclear differentiation is conceived to consist of a loss of specificity in all but the genes that continue to function in the interphase metabolism of the particular differentiated cell in which they reside. The nonspecific properties of genes are their ability to produce the cytoplasmic substances (ribose nucleoprotein) which are the precursors of the substances used in mitosis. The normal differentiated cell thus has a functioning cytoplasmic system, the pattern of which is laid down concomitantly with the differentiation of the nucleus.

In carcinogenesis the observed damage to the normal cytoplasmic constituents [for recent example, see Opie (18, 19)] sets a problem not before encountered in the history of the cell. The new structures must be set up under the influence of a nucleus in which only a few of the genes retain their specificities and hence the cell is no longer able to respond to organizing influences which it formerly obeyed. In the older, less specific terminology a new reaction basis is set up [Durken (20)].

The genes that remain have changed, however, during differentiation and belong to a class which functions predominantly in the metabolism of mitosis when the precursors of growth are present. Once the environment is favorable for division, such a cell proceeds on the course to a malignant tumor. Its three possibilities are to divide, to die, or very rarely to differentiate only into the tissue of origin.

It will be seen that carcinogenesis, via chemical compounds, viruses and physical agents, can be encompassed within the bounds of this hypothesis without making any special assumptions. Further, the loss of specificity [Greene (21, 22)] and the acquisition of autonomy also follow from these premises made initially by Schultz for the purpose of understanding normal differentiation.

The results of Levander and of Lacroix also bear on this subject, as well as on more general pathological growth problems (23, 24). Interested in the problems of the applied art of bone surgery, Levander transplanted bits of periosteum, hard tissue, and bone marrow into rabbits' muscles and varied the usual technic by examining the transplants and their surroundings within one or two days of the operation. He found that the connective tissue cells in the areas surrounding the graft produced fresh bone while the graft perished. As a hypothesis, he considered that the graft exuded some substance which, passing into the surroundings, influenced a specific differentiation in an unspecific tissue. An alcohol extract of bone was then prepared and injected into muscles, whereupon bone and cartilage appeared in 24 per cent of the cases. Extracts of muscle tissue stimulated the production of muscle fibers from mesenchyme. Further work was done, with success, using endometrium. He uses the word "induction" for these phenomena and emphasizes the metaplastic nature of the growth.

Lacroix, also studying bone growth by grafts, concludes that cartilage has a more pronounced inductive effect than had been previously noted. An alcohol extract from the cartilaginous epiphyses of the long bones of new born rabbits led to the production of a large osteoma when the material was injected into the muscles.

It will be noted further that the basis of the Thoma and Goldman classification is not the origin of cells, as is the case in many tumor classifications, but their differentiation and organization. This partly physiological basis of classification, in the authors' opinion, is better than a purely anatomic basis, for the reason that what tumor cells do is more important than what they look like. The names given to many tumors are weird and confusing and there is much argument about names and not behavior in tumor discussions and literature (21, 22). One result of this confusion is the habit of requesting several pathologists to diagnose a particular bit of tissue, keeping secret the diagnosis of one from the other. A difference in names is oftentimes made a lively subject of controversy, whereas in reality the underlying ideas about the diagnosis are the same. Many pathologists hesitate or even refuse to say that a tumor is composed of undifferentiated cells even though they stream out in every direction, are highly polymorphous, polychromatophilic and produce no picture indicative of a dif-

ferentiation. They must give it a name thereby conjuring an illusion of knowledge.

A better example of the possibility of a physiologic diagnosis than the one cited above is presented by tumors of the endocrine organs. The name "granulosa cell tumor" is used by some pathologists for certain ovarian tumors whether or not they give endocrine symptoms. Anatomically they display much variation. It is, of course, difficult and hazardous to venture a physiologic diagnosis on purely anatomic grounds. Whether or not the cells of a given tumor have undergone "chemical differentiation" to the point of producing specific chemical substances cannot be told with certainty by their microscopic appearance. The author suggests that the name "granulosa cell tumor" be reserved for those tumors which produce specific general symptoms and that some other name be chosen for those which look like these but have no such physiologic properties (25, 26). The same remarks apply to tumors of other endocrine organs.

As a final point embracing much of the above, too much rigidity is still assigned in many quarters to the three germ layers. When, for instance, abnormal growths show both connective tissue and epithelial differentiation of their cells, the tendency is to assume that they have been derived from two different sources. Developmental possibilities are greater than developmental fates. Who are we to say at present what protoplasm can or cannot do? For an excellent review, see Oppenheimer (27).

#### PRESERVATION AND SUBSEQUENT TRANSPLANTATION OF SKIN

That cells and tissues can be preserved in viable condition has been known for some years. In the following paragraphs are mentioned a few of the efforts to apply this knowledge to human surgery. Improved methods of treating extensive burns have resulted in a decidedly lower immediate mortality rate (28). As a consequence more patients with extensive denuded areas need skin grafting. There is a persistent hope that skin banks may be developed analogous to blood banks. The difficulties are two: (a) failure of the skin of one individual to "take" when transplanted to a wound of another, and (b) unsatisfactory methods of preservation.

The first difficulty can be ascribed to intrinsic, i.e., genetic factors for want of more precise knowledge. Data are plentiful

on this score from immunological to philosophical sources but are as yet not generally applicable. Briggs' & Jund's results in the transplantation of skin from mouse to mouse may be mentioned (29). Turning the skin grafts around before transplanting so that the hair would grow in a direction opposite to that of the surroundings and thus could not be confused with regenerating host skin, and observing a number of other precautions, their percentage of "takes" in heterozygous strains of mice was no greater than in homoplastic grafts in human beings. When, however, they used inbred strains the percentage of "takes" immediately rose to a reasonable figure. This may account for the fact that occasionally a "take" of grafted skin can be obtained between two human beings.

The second difficulty, that of preservation, is well on the way to solution. The effect of cold upon growth has been studied for many years. Klinker (30) found that both cancer and normal cells remain viable for months after freezing at temperatures to  $-253^{\circ}\text{C}$ . Briggs and Jund (29) slowly froze pieces of ventral skin of three- to five-week-old mice to  $-78^{\circ}\text{C}$ . and rapidly thawed them to  $25^{\circ}$  or  $30^{\circ}\text{C}$ . When transplanted autoplastically, over half of the grafts took. Strumia and Hodge (31) froze forty-one autogenous split-thickness grafts at  $-20^{\circ}$  to  $-25^{\circ}\text{C}$ . for from one to sixty-one days. When transplanted autoplastically to the three patients, 80.5 per cent took perfectly. Among the controls 86.4 per cent took. Within the time limits of the experiments the results of the grafting were not affected by the time of storage of the grafts in the frozen state.

Apparently, then, the freezing method can be used clinically for autoplasmic grafts. Indications for its use should be individualized but include cases in which a number of areas are to be grafted, some of which are ready and others not. Enough skin can be removed at one time to cover the parts ready and those not ready, the skin for the latter parts being preserved for later use. Thus the number of times the patient must be anesthetized can be reduced.

#### GROWTH ABERRATIONS DUE TO ABSENCE OF SOME FACTOR

The growth aberrations due to vitamin deficiencies have been known for a long time and adequately reviewed elsewhere. Results of the absence or deficiency of various other substances, notably

amino acids, have also been recorded, but most of them for the gross effects, i.e., they increased, diminished or had no effect on growth rates, or the animals remained stunted, etc. In the case of a few substances the actual aberrations have been better defined. Of these, the results of labile methyl group deficiency will be singled out for discussion as one of the better known examples. As reasons for this choice it may be mentioned that it is not a compound but a group that is involved, in fact a very simple one. Its effects in promoting normal physiological processes are produced only when it is labile and can be detached, moving around from attachment to attachment. The particular growth disturbance, eventually produced by its absence, is connective tissue hyperplasia in various organs, including the liver which becomes the seat of a certain type of cirrhosis. In additional point of interest is that all forms of cirrhosis of the liver were ascribed for years not to the absence of but to the addition of something such as "toxins," inflammation, and poisons. Perhaps many other conditions now thought to be due to additions will be found included among those already known to be "subtractions," e.g., various forms of anemia.

Some of the events leading to the diagnosis of labile methyl group deficiency are as follows. Animals cease growing when deprived of a source of sulfur as in cystine, cysteine, methionine and to a lesser extent in certain B vitamins. But cystine alone is not capable of growth promotion. Methionine is also needed. Methionine on the other hand can meet the requirements for both cystine and methionine. Therefore, it was concluded and then demonstrated that methionine can be converted to cystine. Apparently cystine cannot be converted to methionine.

Du Vigneaud *et al.*, investigating the mechanism of the conversion of methionine to cystine, prepared homocystine which, if it is to be converted to methionine, must be methylated (32, 33). Even though homocystine had never been isolated from natural sources and may not be a natural intermediate, it nevertheless replaced methionine in the diet for growth support, if B vitamins in crude form (rice, bran extract, and milk concentrate) were also given. The crystalline B vitamins then available (1939) were unable to accomplish what the cruder extracts could do. The livers of the rats in the homocystine-purified vitamin experiments showed extensive fat infiltration and later connective tissue hyperplasia.

Meanwhile, it was known that choline, a nitrogen base with three methyl groups (now considered a B vitamin) had a decided lipotropic action and prevented fat deposits in the liver (34, 35, 36, 37). Methionine had the same effect (38). What is the relation between the chemically unrelated compounds choline and methionine and their physiologically related property of preventing fat deposits and subsequently cirrhosis in the liver? Du Vigneaud seized upon the methyl group as the connecting link especially because homocystine which has no methyl group has the opposite effect on fat deposition. "The possession of a labile methyl group—which choline and methionine had in common was not suspected as the explanation of their lipotropic activity until homocystine-methionine growth studies indicated a metabolic interrelation between choline and methionine" [du Vigneaud 38]. Du Vigneaud then found by labeling with deuterium that choline is synthesized from methionine and vice versa. Transmethylation is now a recognized process.

The biosynthesis of methionine under the conditions of homocystine-choline administration was then demonstrated in rats of an especially well-fed colony (39, 40). This brought to attention the possibility of synthesis of various essentials (in this case "crude" vitamin B substances) by intestinal flora. A method of suppressing bacterial growth in the intestines had been introduced in the form of succinylsulfathiazole (trade name, sulfasuxidine) and when 2 per cent of this substance was administered in a "methyl-free" homocystine diet, growth of the rats ceased in a few weeks (41). When choline was added, however, utilization of homocystine began and growth was resumed. Like effects also followed the giving of a small amount of a specially prepared liver fraction, the active factor of which has not been isolated as yet. Finally, when the succinylsulfathiazole was eliminated from the diet and the rats were returned to the colony food for several weeks, the animals regained their ability to utilize homocystine. These results may be interpreted to indicate synthesis of unknown factors (substances like vitamin B?) by bacteria in the intestines. At any rate, homocystine utilization is influenced. If we adopt the above hypothesis, it can be used to "explain" results such as Popper, György & Goldblatt (42) obtained and as described also by Griffith (43), i.e., when rats were kept continuously on a deficient diet, some overcame the stage of fat infiltration and the



changes due to choline deficiency regressed. Perhaps other processes of equal physiological and pathological import are conditioned by intestinal flora. Interest in these affairs certainly has been manifested for centuries and applied in the buttermilk diet, old age postponement, etc.

To return to the liver: In early experiments there were noted in unstained sections of rats' livers, light greenish-yellow globules of variable sizes and shapes mainly enmeshed in periportal connective tissue. Much effort has been expended in attempting to identify the composition of the globules and trace their origin. Called ceroid, the material at present is regarded as a relatively inert substance, of the nature of a pigment emitting a greenish yellow fluorescence which changes slowly to yellowish white. It probably originates from neutral fat by oxidation of long chain unsaturated fatty acids, though this is not fully established (44, 45).

Emphasis is placed on this ceroid from the present point of view because of questions of its relationship to diet and cirrhosis. Addition of cystine to the cirrhosis-producing diet increased the fat infiltration and the cirrhosis without increasing the amount of ceroid. Choline reduced the fat infiltration and cirrhosis, and no ceroid was produced. Cystine and choline together prevented both cirrhosis and ceroid. It took 40 mg. of methionine in the daily diet to prevent both. Evidently cirrhosis and ceroid are not necessarily connected, but ceroid deposition apparently requires antecedent changes in fats and particular kinds of fat in the diet (cod liver oil).

No ceroid was found in over two hundred human livers either normal or at the site of cirrhosis; only diffuse necrosis and hemo-chromatosis were found. Russakoff & Blumberg report that seven of nine patients with uncompensated portal cirrhosis improved on the addition of choline to a high protein diet. They remark that it is not to be expected that the connective tissue once proliferated in the liver would disappear but its further growth might be arrested (50). Regression occurred in rats' livers on adding cystine and choline to their diet but a residue was left attesting to arrested cirrhosis (46, 47). The above findings are not restricted to rats but similar conditions have been found in rabbits and dogs (48, 49).

From the foregoing it seems then that the absence of free methyl groups in the diet is directly concerned with connective tissue overgrowth in the liver and that the addition to the diet of substances supplying this group in available form can arrest the proc-



ess and allow a certain amount of recovery. The ability of the liver to regenerate is too well known to require more than mention.

To the question of whether, in the presence of sufficient free methyl groups in the diet, there are metabolic blocks within the organism so that they cannot be properly utilized there is no answer at present. The addition of certain substances, notably carbon tetrachloride, produces cirrhosis which may be counteracted by sulfanilamide. Does carbon tetrachloride act via methyl groups? In alcoholic cirrhosis, the viewpoint as to etiology has undergone radical change in the last years. The consumption of alcohol reduces appetite, less food is consumed and what is eaten is improperly chosen. Hence alcoholic cirrhosis is really dietary cirrhosis. Captive wild animals (specimens in Laboratory of Pathology of Philadelphia Zoo) develop cirrhosis of the liver similar anatomically to human "alcoholic" cirrhosis without ever having had a drink of alcohol.

György & Goldblatt (51) found that 0.1 per cent thiouracil in a cirrhosis-producing diet has a preventive effect on the production of cirrhosis in rats. They call attention to the effect of thiouracil in inhibiting thyroxine production by the thyroid gland. Can the organism liberate methyl groups, under certain conditions, from compounds in which they ordinarily are tightly bound?

Further discussion would lead too far afield. To repeat several principles: Labile methyl group deficiency and cirrhosis of the liver are clear examples of the pathological sequences which can arise by devious and complex paths from a fundamentally simple chemical agent. It is further an example of the effects of the subtraction of a factor and not the addition of some nonnaturally-occurring substance.

#### SPECIFIC GROWTH-INFLUENCING SUBSTANCES

The literature contains accounts of many substances having influences on various phases of growth, some accelerating, some inhibiting, some acting directly and others indirectly through various physiological mechanisms. Intense practical interest centers around them in such fields as wound healing, regeneration, replacement, and especially the inhibition and destruction of tumors. A few have been applied in the latter field in the form of "leads" but very few systematic studies have been made in what might be called the "chemotherapy" of tumors. None is compar-

able in effect to the planned studies in the therapy of infections. Obviously, if something is to be discovered for the treatment of cancer, it must be done experimentally. To do it requires teamwork of a high order of efficiency. The work must be founded on expanding knowledge of differential growth requirements, differential susceptibility and response, differential enzyme patterns and many other branches. As examples of knowledge bearing on selective inhibition of one tissue or one phase of the life of a tissue, in preference to another tissue or another phase in the life of the same tissue (mitotic phase, etc.), due to differential susceptibility, several papers may be mentioned. Thus the work with inositol and folic acid bears on the problem (52). Emetine was first tried in cancer patients in 1918 (53). Medawar's differential mesoderm inhibitor (54) obtained from such natural sources as malted grains, yeast, and the ripe berries of the mountain ash, *Sorbus*, led to the "synthetic differential growth inhibitor" of Medawar, Robinson & Robinson (55). It is similar to panto-lactone which combines with beta-alanine to form pantothenic acid. Kuhn & Jerchel synthesized an unsaturated delta hexenolactone and found it structurally identical with natural parasorbic acid from *Sorbus* berries (56). It has the same physiological properties as the natural acid, viz., it inhibits fibroblasts at concentrations which do not affect epithelium. Hauschka confirmed this finding in the flatworm *Dugesia tigrina* and discussed the biochemical implications with Toennies & Swain (57, 58). The results were not as clear cut in tissue cultures. Royle (59) found no differential effect in cultures of stomach and intestine, mesonephros and a human breast carcinoma. Briggs, using frog embryos, found irreversible damage in concentrations of hexenolactone sufficient to stop cleavages. Since it was observed that the vegetal hemisphere is more sensitive to hexenolactone than the animal and that nuclei divided while the cytoplasm was inhibited from cleaving, the substance may serve as a tool for further studies on cell physiology (60).

Differential enzyme patterns have been studied by Greenstein, *et al.* among others. It seems that the patterns in different malignant tissues resemble each other much more closely than the patterns of various normal tissues. Apparently no application of this finding has been made to date except as a useful basis for constructing correlating hypotheses from various fields (61, 62).

Among the "leads" actually tried in the chemotherapy of malignant growths of animals, mostly mice, mention is made of a few; and several, which in addition have been tried in human beings, will be described. The colchicine molecule has been altered to reduce toxicity and still retain its well-known effects on mitosis (interference with spindle formation (63, 64, 65). Cacodylates have been tried. Williams (66) tried suramin (Naphuride, Winthrop), the azo dye chlorazol fast pink, histamine, and depropanex (deproteinized pancreatic extract) on transplanted mouse lymphosarcoma. The idea was to discover whether anticoagulants and vasodilators would cause metastasis of the transplanted tumor beyond adjacent lymph nodes in degrees greater than in the controls. No particular success was achieved.

Many years ago it was noted that occasional patients with advanced malignancy (carcinoma and more particularly sarcoma) recovered from their tumors after an acute bacterial infection, chiefly erysipelas. William B. Coley, observing such a case about 1891, devoted much time and attention for the rest of his life to the phenomenon (67) and showed that the bacterial toxins were effective. The source of the first toxins was *S. erysipelatis*, and the fluid containing the toxin was called Coley's fluid. A year or two later, toxins from *B. prodigiosus* were also found effective following the work of Roger (68). It is now known that the toxins of *B. prodigiosus* are more potent in destroying tumor cells than those from various streptococci. From then on numerous preparations were made here and abroad, varying in therapeutic results. Dosage was determined by febrile reaction (69, 70). About 1933, Shear (71) began fractionating the material from *Serratia marcescens*. The bacteria were grown on a simple medium of three inorganic salts and a small amount of glucose. The active fraction was found to be a substance of the nature of a polysaccharide containing about 2 per cent nitrogen. A dosage of 0.1 microgram produces hemorrhage and necrosis in 50 per cent of the transplanted mouse sarcomas. A dose, sufficient to completely destroy large mouse tumors, kills a large percentage of the mice. Those which recover usually remain free of the tumors which ulcerated and sloughed out. Doses corresponding to the amounts needed to "cure" mice have not been used in human beings.

The polysaccharide is highly toxic and is better studied in man than in animals. Within an hour after intravenous injection

of 0.001 mg. of the polysaccharide, fever appears which increases to a maximum in four to eight hours, reaching 105°F. or more. The temperature then slowly returns to normal, usually in twenty-four hours, but sometimes low grade fever persists for several days. Many patients complain of pain in the region of the tumor beginning in an hour or two and lasting for several days. When the tumors are close to the surface, ecchymosis is likely to appear in a few days. There seem to be no untoward effects on kidney function or heart action immediately or later, or on general well being. With the rise in temperature, there is a coincident fall of blood pressure to 70 mm. Hg., or less, which on treatment by ordinary shock therapy returns close to the level before treatment in twenty-four or forty-eight hours. The same necrosis and hemorrhage, as are demonstrated in transplantable mouse sarcomas occur in human sarcomas. Considerable detail of trials on human beings was given in papers presented at the Gibson Island meeting in the Summer of 1945 and at the meetings of the American Association for Cancer Research, May, 1946.

The material produces a type of immunity, and repeated doses are usually less and less effective on the tumor cells. The mechanism of immunity is under investigation. It is not exactly a Schwartzman phenomenon inasmuch as a sensitizing dose is not necessary for the hemorrhage and necrosis to occur, but Schwartzman himself has studied the mechanism (72, 73). While in no sense is this material a "cure" for cancer, it nevertheless does destroy some cancer cells and has yielded interesting results in the field of cytology.

The polysaccharide acts on cells in division, destroying them, and the hemorrhage occurs simultaneously with necrosis, not so much from destruction of endothelial lining of vessels as from necrosis of surrounding cells. How cells in mitosis are destroyed is an open question, but changes in permeability are observed to occur. The hemorrhage is most spectacular in sarcomas, probably because the latter are generally more vascularized than carcinomas. This is consistent with the clinical observation that occasionally a carcinoma has regressed after erysipelas or after administration of Coley's fluid.

Another group of substances, the carbamic esters (urethanes) inhibiting growth, have been studied by Haddow & Sexton (74). These substances, called caryoclastic poisons (75, 76), have an

effect upon the mitotic cycle. Haddow & Sexton chose ethylphenol carbonate and various derivatives for special study. Considerable literature exists on the action of urethane on growth processes in a variety of animals and plants. The substance first produces a transient increase of the mitotic count in the intestinal epithelium of the mouse, not the same as that produced by colchicine. It also retards the growth of spontaneous mammary cancer in the mouse and Walker rat carcinoma 256. The action of urethane upon the growth of the Walker carcinoma was found to be accompanied by a change of the histological picture from a cellular to a more fibrous type of structure. The authors have the impression that it is an attempt at further differentiation on the part of the cells. Readers need no more than to be reminded of the antagonism between proliferation and differentiation, a phenomenon known for many years and commented upon in all text books of biology. [Weiss, for instance, gives illustrations of this principle.] In the field of general tumor pathology it has been emphasized for many years and is the basis for the histological grading of human tumors, a procedure of statistical value but not useful for individual cases if the criteria are taken by themselves as an index of prognosis. Haddow & Sexton attempt explanation of the action of urethane in this connection but much work remains to be done. Chemically, they consider relations to dehydrogenases, flavoprotein, the purines, and various other chemical substances.

Patterson, Haddow, Thomas & Watkinson report on the treatment of leukemia with urethane following the previously mentioned discoveries (77). The total dosage was determined by the tolerance of the patient and by a diminution in the number of circulating white blood cells. It varied from 19 grams to 134 grams in thirteen patients who had no x-ray subsequent to the administration of urethane. There was a tendency for the differential count to approach a more normal pattern as the total number of circulating white blood cells was reduced. The size of the spleen diminished and the lymph nodes become smaller. The authors state that the results are "remarkably similar to those produced by standard methods of deep x-ray therapy." Relapses took place but the time of treatment has been too short for any statement to be made upon the length of life. In thirteen cases of advanced carcinoma of the breast and eleven cases of various other malig-

nancies, there was temporary diminution in the size of the lesions in a few of the cases.

The nitrogen mustards are injurious to tissues, especially rapidly growing ones. They are toxic, require great care in their trials, and, especially in the slower growing forms of lymphomatous growths, produce regression lasting for variable but relatively short times. In a report on their trial in neoplastic disease, Rhoads (78) summarized results on 160 patients chiefly with lymphoma, leukemia and allied conditions.

Finally we mention the work of Roskin (79) who injected mice bearing Ehrlich carcinoma with *Schizotrypanum cruzi* and found recession of the tumor in thirty of forty-five test animals, while in forty-five control mice the graft grew. The infection finally killed the mice in all cases. To determine the specificity of *S. cruzi*, *S. duttoni* and *T. equiperdum* were used in other mice and found to have no effect on their tumors. Endotoxins were then prepared from *S. cruzi* and found to inhibit and cause regression. In three patients with cancer of the pharynx, endotoxin treatment was encouraging. In his summary, Roskin states that "toxin therapy may become one of the methods for treating malignant tumors."

The foregoing descriptions we believe are sufficient evidence to justify the statement that there are, at least, a few leads which merit further study on the pathology of growth.

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THE LANKENAU HOSPITAL RESEARCH INSTITUTE  
AND THE INSTITUTE FOR CANCER RESEARCH  
PHILADELPHIA, PENNSYLVANIA

## DEVELOPMENTAL PHYSIOLOGY<sup>1</sup>

BY ALBERT TYLER

*William G. Kerckhoff Laboratories of the Biological Sciences  
California Institute of Technology, Pasadena, California*

The present review deals mainly with the earlier stages of development and attempts to assemble most of the relevant work that has appeared since Hamburger's (3) summary [see also 1 and 2] as well as to include some of the older work from foreign journals which are again arriving in this country. In this country an excellent new text by Brody (9) on growth [reviewed elsewhere (10)] contains much material of embryological interest. From abroad three books of great interest are now available: one by Dalcq (4) contains a very thorough survey of the field of experimental embryology, a fine treatise by Brachet (5) [reviewed elsewhere (6, 7)] covers the more chemical aspects of this field, and the third, by Lehmann (8), is a comprehensive treatment of the experimental work on amphibians and sea urchins, which has appeared mainly in the past fifteen years. There is also a brief account of work in experimental embryology which has been carried out in Holland during the war years [Raven (11)].

### MICROTECHNIQUES

Experimental embryology has reached a stage where investigation of the metabolism of minute amounts of tissue and tissue cultures will evidently contribute greatly to the solution of its problems. One technique that promises to be of great use for this purpose is Linderstrøm-Lang's ingenious method of measuring gas exchange by application of the principle of the Cartesian diver (12, 13, 14, 15). However, it does not obviate the need for the development of simpler devices that permit easier manipulation and concomitant microscopic examination of the tissue. Microchemical techniques recently developed make possible the titration of fats (16), determination of their iodine number, extraction and fractionation of lipids (17), determination of total nitrogen content (18), and the determination of pentoses in 30 to 50 mg. of tissue (19). Extensive work has been done on cytochemical methods and,

<sup>1</sup> This review covers the period from June, 1945 to June, 1946 and includes earlier work not previously reviewed.

although these cannot be present here, reference should be made to the review by Dempsey & Wislocki (20) of the contributions of such methods to physiology, to the extensive use made of them in studies on the placenta by Wislocki *et al.* (21), to Brachet's (19) method of detection of pentosenucleic acids, and to Pollister & Mirsky's (22) method for localization and determination of protein in the presence of nucleic acid. At the same time mention should be made of recent criticisms (23, 24, 25) that have been leveled at the indiscriminate use of cytochemical methods and the difficulties inherent in them. For manometric respiration studies involving the use of cyanide, a new series of calcium cyanide-calcium hydroxide mixtures to be used in the center well of the vessel to maintain constant cyanide tension and permit adequate absorption of carbon dioxide for fluids containing up to  $10^{-2}$  M HCN is presented (26).

#### THE SPERMATOZOÖN

*Structural features.*—Electron microscope studies on the spermatozoa of man (27), bull (28), squid (29) and sea-urchin (30) demonstrate that the axial filament of the tail is composed of a number (usually 9 to 11) of subfibrils which are seen most readily in the exposed endpiece after treatment of the fresh sperm with distilled water. In much of this preliminary work no particular pains have been taken to fix the material. To the reviewer, from personal experience, the use of proper fixatives seems at least as important here for the maintenance of normal appearance as it is in cytological studies with the light microscope.

Embryologists as well as geneticists have long been interested in the arrangement of the chromosomes in the sperm. Direct observational evidence (31) with the light microscope now shows that in three species of iceryine coccids the chromosomes are in linear alignment, attached end to end. Abnormal spermatogenesis with the elimination of chromosomes occurs frequently in many species of animals. In one of the pentatomid Hemiptera, *Brachystethus rubromaculatus*, Schrader (32) has described, as a regular occurrence in the fourth lobe of the testis, the formation of small sperm containing only X or Y chromosomes and giant sperm containing all the autosomes which have passed as undivided tetrads to one of the four spermatids. From his studies, Schrader concludes that the giant sperm enter the egg and suggests that, while they

do not participate in development in an hereditary sense, they may have some beneficial action by supplying large amounts of nuclear material to the developing egg. In a brief report by Pollister & Lavin (33) on atypical spermiogenesis in *Viviparus malleatus*, in which chromosomes entering the larger cell at the unequal second division subsequently degenerate, evidence is presented for the conversion of the desoxyribonucleic acid of the chromosomes into ribose nucleic acid of the cytoplasmic granules. A note (34) on the chemistry of fish sperm, as well as the nuclei of other cells of vertebrates, points to the presence in nuclei of an acidic protein, called chromosomin, which is considered to be an essential part of the chromosomes.

Since in animals with male heterogamety the two kinds of sperm differ in their sex chromosomes, methods of separating the sperm for control of sex have often been proposed. Harvey (35) suggests centrifuge methods for accomplishing this with mammals, presents calculations showing the definite possibility of success, and indicates the refinements of technique necessary.

*Functional features.*—The metabolism of mammalian sperm is now known to be predominantly of a glycolytic character. In the absence of glycolyzable sugars, the oxidative utilization of phospholipid reserve evidently furnishes energy for motility. Lardy & Philips (36) have shown that the sperm and enzyme preparations from them can, under certain conditions, utilize citrate, isocitrate, *cis*-aconite and the four-carbon dicarboxylic acids of the Krebs' cycle, can form citric acid from such metabolites as pyruvate and oxalacetate, and that fat oxidation by sperm is inhibited by malonate. This is taken to mean that fat, as well as carbohydrate, is oxidized stepwise through the isocitric acid cycle. Sperm have also been shown (37, 38) to contain a complete cytochrome system and adenosinetriphosphate (ATP). The ATP and cozymase act as coenzymes in several intermediary reactions in the sperm glycolysis and evidence is presented (38) to support the view that ATP is the substance through which a link is established between the activity of sperm and glycolysis. In the sea-urchin it has been shown (39, 40) that sperm retain their fertilizing power longer and the usual decrease in rate of respiration is retarded, when diluted with seminal fluid rather than with sea water. In the terrestrial isopod *Armadillidium vulgare* (41), sperm are found to survive more than a year in the female genital tract.

When ice is applied to the scrotal testes or epididymis of a rabbit for 10 minutes, separation of head and tail occurs in about 50 per cent of the spermatozoa in the ejaculate. Tests (42) of the fertilizing capacity of the sperm from "iced" testes show that most of the remaining sperm are nonfunctional. There is, however, no differential effect on male and female producing sperm, the sex ratio of the offspring remaining normal.

In a study of sperm transport in the rat it has been shown (43) that the ejaculate from males whose vesicular and coagulating ducts were ligated does not pass through the cervix uteri. The formation of the copulation plug evidently is important in enabling the sperm mass to enter the cornua. In another study (44) on sperm transport, hyaluronidase has been demonstrated in the uteri of rabbits immediately after coitus. Since the concentration of hyaluronidase decreased with time, it is concluded that the spermatozoa probably do not secrete the enzyme while in the female genital tract. However, this conclusion seems premature since there are various other possible interpretations of the decrease, such as combination with uterine tissue or fluid and deterioration of the liberated enzyme.

#### THE UNFERTILIZED EGG

*Growth and ovulation.*—Painter (45), considering the facts that the nurse cells of the ovary are highly polyploid and are absorbed by the egg during oögenesis in the fruit fly, has proposed the notion that the chief function of the nurse cells is to supply the egg with a large quantity of nuclear material which could be readily assimilated by the rapidly dividing nuclei of the zygote. Somatic polyploidy is evidently quite common in arthropods, and Montalenti (46) reports finding cells with giant nuclei in the testes in several species of isopod crustaceans. For these the original theory of Painter would not apply, and he suggests that the function of these cells is to elaborate the secretion that accompanies the sperm. This would accord well with the recent views of Painter (47) relating endomitotic growth with protein synthesis and secretion. He has also suggested (45, 48) that endomitosis occurs in the growth of the germinal vesicle of the oöcyte along with a form of chromatin diminution in which heterochromatin is detached from the chromosomes in the form of small granules of desoxy-ribose nucleic acid and appears as ribose nucleic acid in the cyto-

plasm where it may play a role in the elaboration of the yolk. However, Ris (49) interprets the "lamp-brush" chromosomes of the germinal vesicle as typical diplotene chromosomes in which there is a tremendous longitudinal growth of the chromonemata with loops representing the major coils of the laterally separated strands. In salamanders observations on leucocytes present in ovarian eggs lead to the view that lymphocytes are concerned in the growth of the egg while neutrophils and eosinophils are involved in its involution (50). A quantitative study (51) has been made of oöcyte production and growth in the rat.

Following up investigations with vitally stained Golgi bodies, Worley (52) has studied vitellogenesis during oögenesis in the mussel and reports that the primary oöcyte is apparently devoid of formed protein yolk, but loaded with fat droplets formed by the Golgi apparatus. In the snail *Limnaea* the oöcyte stages are reported (53) to be devoid of thymonucleic-acid-protein which is present only in the chromosomes of the oögonia and of the ripe egg at the maturation divisions.

A new enzyme, a phosphoprotein phosphatase, has been reported (54) to be present in ovarian eggs of the frog. Dipeptidase determinations have been made (55) on the nucleus and the cytoplasm during growth of the frog oöcyte. None is found until after yolk formation begins. The enzyme content of the nucleus rises in general with increased nuclear volume, but the rise is not directly proportional to that of the cytoplasm.

Hens have been made (56) to ovulate as much as thirty hours prematurely by the injection of anterior pituitary hormone. Coelomic eggs of a salamander give, about 28 to 38 per cent fertilization if covered with jelly from the oviduct before insemination, and only two to three per cent without the jelly (57). The jelly appears to aid the sperm in its penetration of the egg. Development is usually abnormal. Claims of *in vitro* ovulation obtained by means of pituitary extracts in the frog have been disputed (58) and convincingly reaffirmed (59).

*Structure and composition.*—Extensive investigations have been made by Runnström & Monné (60 to 66) on the structure of the sea-urchin egg with special reference to the surface layers. They recognize four such layers—the gelatinous coat, the vitelline membrane, the luminous layer and the cortical gel, the latter two together being also termed the cortex or cortical layer. The

vitelline membrane shows (60) no marked birefringence but upon formation of the fertilization membrane birefringence increases to a maximum at full elevation. The cortical layer shows positive birefringence in the radial direction with no change upon fertilization. Treatment with lipoid-liquefying agents lowers considerably the birefringence of the cortex which may, therefore, be due chiefly to oriented lipoid layers. The unfertilized egg shows a wrinkled surface upon shrinkage in hypertonic sea water, but after treatment with trypsin the eggs shrink with a smooth surface. Also they do not form a fertilization membrane. It is tentatively concluded that the vitelline membrane is composed of elongated protein molecules cemented together by lower molecular weight substances which separate from the membrane upon fertilization. The protein molecules then assume a tangential position with new firmer bonds between them. The gelatinous coat of the egg shows (64) no birefringence under ordinary conditions but when stained with basic dyes, it becomes positively birefringent in a radial direction. Crystal formation in the nucleus under the action of hypertonic sea water has been studied by Monné (65) who presents evidence to show that in the oöcytes the crystals are chiefly protein, while in the mature eggs they are mainly lipoids attached to a small amount of protein. He proposes (66) a fibrillar view of the structure of the ground substance of the cytoplasm. Monroy & Oddo (67, 68) have confirmed the observations relating to the birefringence of the cortex and support the view of its lipoid nature. Various tests lead them to regard the cortex as a liquid crystal.

The distribution of nucleic acid between sedimentable (by ultracentrifuge) granules and supernatant of frog eggs has been examined by Brachet & Chantrenne (69) who report that about half of the nucleic acid is associated with the granules in the very young oöcytes. The amount decreases to between twenty and forty per cent at the end of oögenesis. By contrast most of the nucleic acid in the tadpole tissues, and almost all of it in the adult tissues, is in the granules. Investigations on a variety of vertebrate tissues and on yeasts (cf. 70 to 73) show a number of enzymes and hormones to be associated with these granules which are considered to play a role in the synthesis of proteins involved in growth. Further studies on the enzymes of the nucleus and cytoplasm show (74) that arginase is not detectable in nucleus or cytoplasm of ripe

or of immature oöcytes, that ribonuclease is present in both nucleus and cytoplasm in approximately equal concentration, and that only slightly more alkaline phosphatase is present in the nucleus than in the cytoplasm. Thus these enzymes, as well as other hydrolases, which have been presumed to be involved in nucleoprotein synthesis, are evidently lacking, or at least not particularly concentrated, in the nucleus. Experiments and observations of broad scope have been made by Holtfreter (75, 76) on the "lipochondria" of the amphibian egg. These he describes as granular bodies interspersed among the yolk platelets throughout the egg and containing lipids surrounded by a layer of protein, the removal of which permits coalescence of the lipid droplets to form "liposomes." Such a transformation takes place also in tissues of the young larva where it appears to be associated with the phenomenon of yolk resorption. Various properties of these bodies have been studied, and myelin-formations and Golgi systems have been produced from the liposomes or extracts. An investigation (77) of the egg-yolk proteins of a cat-fish has yielded two new proteins—a typical ichthulin and an albumin.

In studies of physical properties Howard (78) has conclusively shown that the freezing point depression of the hen's egg yolk is equivalent to that of the white and 25 per cent less than that of chicken blood. Harvey (79) has examined the stratification and breaking of sea-urchin eggs in various single salt solutions and shows that ease of breaking is not determined by effects on interior viscosity but on the surface layers. Raven & Klomp (80) have studied the osmotic properties of eggs of a pond snail.

#### FERTILIZATION

*Specific interacting substances.*—There has been a revival of interest in F. R. Lillie's fertilizins and antifertilizins which Hartmann has termed gynogamones and androgamones. Their presence and testing methods for them have been reported in additional species of sea-urchins (81), mollusks (82), and for the first time in vertebrates, in lampreys (83, 84) and trout (85). Starfish fertilizin which does not ordinarily agglutinate species sperm can be made (86) to do so by the addition of certain adjuvants. This discovery goes far towards accounting for the apparent absence of fertilizins in many species of animals. The protein nature of these substances has been confirmed and additional chemical data (87, 88) show



sea-urchin fertilizin to be a highly acidic, polysaccharide-containing protein of low nitrogen content with elongate, gel forming, molecular structure. Antifertilizin is also an acidic protein, but evidently of low molecular weight. A new agent, having a lytic action on the surface of the unfertilized egg, has been discovered (88, 89) in methanol extracts of sea-urchin sperm. This may be related to the egg-membrane lysins of molluscan sperm and the hyaluronidase of mammalian sperm, but judging from the method of extraction its chemical properties appear quite different. The immunological approach (90) to the interactions of these substances has continued with the demonstration (91) that guinea-pig complement, which is bound by sea-urchin fertilizin, is released by the action of antifertilizin. It has also been found (92) that the fertilizing capacity of sea-urchin sperm can be greatly reduced without loss of activity by treatment with "univalent" antibodies obtained by immunization of rabbits with purified antifertilizin and photooxidation (93 to 96) of the antiserum. Questions of specificity of the sperm and egg substances continue to arise especially in view of the wide cross-reactivity that has been obtained (87) with some of the preparations. Investigations of the heteroagglutinins, of broad group-specific activity, found (97 to 99) in body fluids and sperm extracts of various species of invertebrates may help establish a basis for understanding the specificity problem. The fertilizin work has also led to an auto-antibody concept of cell structure and growth which further evidence (100) tends to support.

The highly interesting work on hyaluronidase continues with the report (101) that a nonfertilizing quantity of sperm gives successful fertilization upon artificial insemination of rabbits when it is supplemented with sperm extracts containing this enzyme. Other tests (102, 103) show that the follicle cells of ova of rats that had been immunized to hyaluronidase are dispersed quite as readily as those from non-immunized rats. Antiserum, however, inhibits the action of the enzyme *in vitro*, as does also, to a lesser extent, normal serum of rats and of certain other animals. Specific interacting substances are quite likely involved in the mechanism of self-sterility in animals like Ciona, and further work (104) on this problem perhaps points to them as part of the explanation of the increased self-fertilization obtained with increasing amounts of sperm.

*Chemical and physical changes.*—In a brief review (105) of some experiments relating to the physiology of fertilization it is suggested that electrostatic patterns on the surfaces of egg and sperm may account for the specificity of their union and the block to polyspermy. Upon fertilization of the sea-urchin egg there is a breakdown of carbohydrate (mainly of glycogen) evidently (106) through phosphorylated intermediary products by means of oxidative decarboxylation, and an approximately equivalent production of an acid. Both the carbohydrate breakdown and acid production are completely inhibited by phloridzin but not by sodium fluoride nor monoiodoacetate. According to the evidence (107) free glycogen is first linked to protein, then broken down to phosphogluconic acid and successively degraded by oxidative decarboxylation. A difficultly hydrolyzable phosphate ester, 1,2-propamediol phosphate, has been found (108, 109) in sea-urchin eggs and isolated from beef brain. It has a strong activating influence on the carbohydrate breakdown and results in the formation of pentoses. It is concluded that the muscle form of metabolism involving trioses is of minor importance in the changes upon fertilization, but that Dickens' oxidative decarboxylation scheme preponderates. It has also been reported (110) that fat is oxidized after fertilization but this is disputed (107). A marked increase of free cholesterol is found (111) to occur as early as ten minutes after fertilization in the sea-urchin egg while the total cholesterol content remains constant (110, 111). Of considerable interest is the demonstration (110) of a change in the solubilities of cephalin and lecithin in ether and alcohol occurring upon fertilization. It is suggested (107) that this is due to binding with protein.

Sea-urchin eggs of the genus *Arbacia* possess a red pigment called echinochrome (112 to 115). Upon fertilization a release of this pigment is reported (116) to occur. Other changes occurring upon fertilization that have been investigated recently include viscosity and electrical conductivity of sea-urchin egg brei (117), and formation of protoplasmic cones (118) and ameboid movements (119) in eggs of snails. New mechanisms proposed for membrane elevation in the sea-urchin (120) and starfish (121) involve the incorporation of the cortical granules of the unfertilized egg into the fertilization membrane. The redox potential of the fluid between packed unfertilized and fertilized eggs and sperm of *Arbacia*, *Asterias*, and *Chaetopterus* has been measured (122) and

activation interpreted as the result of bringing the egg to the proper redox level.

*Artificial activation.*—Work on artificial parthenogenesis that has appeared since the last general review (123) includes:—an attempt (124) to apply the "sensitization" theory to sea-urchins in which calcium has no influence on activation, the demonstration (125) of a sensitizing effect of picric acid on *Nereis* eggs, and an inhibitive action of acetyl choline (126) on the activation of sea-urchin and *Urechis* eggs. In addition spontaneous incipient parthenogenesis is found (127) to occur in a small per cent of rat ovaries and to be uninfluenced by treatment of the animals with gonadotrophic or sex-hormones.

#### CLEAVAGE

Critical evidence of the morphological reality of spindle fibers is given in observations (128) on cleaving eggs of a mite in which the primary, continuous fibers may be seen with ordinary light and no complicating astral rays are present. Evidence is summarized (129) in favor of traction fibers which draw the chromosomes to the poles. In a review (130) discussing changes in nucleic acids during cell division, it is suggested, on the basis of their presence in the microsomes along with various enzymes, that they may serve to cement together the enzymes necessary for the synthesis of proteins. Penicillin (131) and caffeine (132) are found to inhibit cleavage in the sea-urchin egg, the former with no effect on the rate of oxygen consumption, the latter with an approximately proportional reduction. Syncytia which may occur spontaneously in *Ciona* eggs as a result of disappearance of cleavage furrows, or their failure to form, show (133) almost as great a rise in respiratory rate during development as the controls. This again shows that the formation of cell boundaries is not essential for the rise. Colchicine injected intraperitoneally is found (134) to inhibit the cleavage of mouse eggs *in vivo*. Colchicine, various quinones and stilboestrol are found (135 to 137) to block mitosis at different specific phases in *Tubifex* eggs. The accelerating action of optimal crowding on rate of cleavage, previously reported in sea-urchins, is extended now (138) to frog eggs. Viscosity has been followed (139) by the Brownian movement method in different parts of dividing grasshopper neuroblasts, and it is suggested that alterations in water and nucleic acid content may

account for the changes observed. In the sea-urchin the positive double refraction of the cortex of the unfertilized egg is found (140) to disappear upon fertilization and reappear temporarily at anaphase of the first cleavage. Unilateral ultraviolet irradiation of unfertilized sea-urchin eggs (141) causes the first cleavage plane to pass almost invariably through the center of the irradiated region.

#### EMBRYONIC METABOLISM

A review (142) of work up to 1941 on the metabolism of the sea-urchin egg attempts to give physico-chemical expression to the "double gradient" hypothesis of Runnström, principally on the basis of the inhibiting action on specific metabolic processes of various chemical agents that influence differentiation in a predominantly animal or vegetative direction. Child's gradient hypothesis, is criticized (143) particularly on the basis of the similarity of respiratory rate of animal and vegetal halves of early sea-urchin blastulae. Child (144), in turn, launches a vigorous attack on the "organizer" concept and suggests a more generally applicable view of mutual interactions in development based on a revised gradient hypothesis. Another theoretical paper (145) attempts to account for regulation in development on the basis of physiological competition of embryonic parts.

Measurements (146) of the respiration and cytochrome oxidase content of brei prepared from frog eggs and embryos support the view that the spatial relations of enzyme and substrate, rather than enzyme or substrate synthesis account for the rise in rate of respiration during development. In *Amblystoma*, measurements (147) over relatively longer periods of development show a steady rise in cytochrome oxidase activity and the rise in respiratory rate is attributed to transformation of yolk into metabolically active material. The absolute rate of respiration at any given stage is not assumed here (147) to be limited by cytochrome oxidase activity, although, as in the frog (146), the respiration is considered to be mediated largely by that system. The metabolism and distribution of ribonucleic acid in amphibian embryos is discussed in a brief review (148) relating it to the normal process of neural induction. Contrary to the hypothesis of the liberation of "masked evocator" by glycolysis, it is found (149) that glycogen disappears from cells that turn in at the ventral blastoporal lip as well as at

the dorsal lip, but not in cells of explants performing induction. Grasshopper embryos are the subject of further metabolic studies in which changes in carbohydrate (150) and uric acid (151) are followed. Included in these studies are: (a) additional evidence in favor of the sequential utilization of carbohydrates, fats, and proteins during development, (b) an estimated breakdown of 6.6 per cent of the initial protein and (c) evidence for extensive inter-conversion between free sugars and higher carbohydrates. In the sea-urchin the total lipid content decreases during early development (110) and a comparison with data on respiration shows this to be consistent with a combustion of fat supplemented by increasing breakdown of carbohydrate. The respiratory rate of echinoid eggs is found (152) to be depressed in concentrations of lithium chloride compatible with development but, in contrast to previous reports, the increment in rate is found to persist. Halves of *Ciona* eggs obtained by separation in the two and four cell stages show (153) no significant differences in respiratory rate despite the well-known differences in developmental capacities.

Many parasitic nematodes are well adapted to relatively anaerobic conditions. In a fish parasite *Eustrongyloides ignotes* which leads a more or less completely aerobic life, the larvae are found (154) to survive for long periods in almost, but not quite, complete lack of oxygen and to consume very much more of their reserve glycogen. *Taenia* larvae have (155) a cyanide-sensitive respiration and are able to reduce methylene blue anaerobically in presence or absence of glucose.

Studies have been made of the pH of the yolk of developing eggs of six species of birds (156) and of the pH and Eh of the allantoic fluids of normal and influenza-infected hen's eggs (157). The metabolism of mineral substances and of water has been studied extensively in amphibia (158 to 163) in salmon (164) and in the sea-urchin (165).

#### EXPERIMENTAL MORPHOGENESIS

*Polarity and symmetry relations.*—Harrison (166) attempts to interpret the determination of polarity and symmetry in embryos in terms of modern concepts of crystal structure, using experiments (hitherto unpublished for the most part) on the internal ear of the amphibian embryo. Both adjacent medullary cord and underlying mesoderm can induce ears in indifferent ectoderm al-

though presumptive ear ectoderm of the early neurula is to some extent capable of self-differentiation. At first the ectoderm is isotropic about an axis perpendicular to the surface, then the antero-posterior axis is established in the late neurula and later the dorsoventral axis. In the period of monaxial determination fragments of the ear rudiment may form a whole ear as may also the fusion of two ear plates. Harrison regards these phenomena as expressions of forces involved in the molecular configuration of the egg protoplasm, particularly the orientation of the protein molecules in the cell. A theory to account for the segregation of oöplasmic constituents is advanced (167) on the basis of Teorell's "diffusion effect," which consists in the establishment of a potential by the diffusion across and within a membrane of an electrolyte, having ions of different mobility, whereby an accumulation or impoverishment inside of other anions or cations is produced.

Tests of the effect of dinitrophenol on the polarity of *Fucus* eggs show rhizoids to form on the side of higher concentration in steep gradients across cells imbedded in thin agar or gelatin films (168). Further experiments (169-172) on bilateral symmetry in the frog embryo show that, while the entrance point of the sperm is normally the determining factor, this can be overcome by directed rotation of orientation of the egg, effected by causing the eggs to adhere to the substratum by their gelatinous coat in any desired position. In an analysis (173) of asymmetry the development of the digestive tract of amphibia, the production of situs inversus viscerum by experimental procedures, such as rotation and extirpation of parts, is shown to depend upon the extent of compensating influences of the intact regions. It is concluded that asymmetry is established by a secondary displacement of certain growth substances distributed in a regular manner along the main axis of the embryo. Experiments (174) on reversing the medullary plate in amphibians show that the antero-posterior but not the lateromedial axis of the medullary ectoderm is established in the early neurula. In rats, upon rotation of segments of the uterine horns through  $180^{\circ}$  prior to impregnation, the embryos are found (175) to be oriented correctly with respect to the displaced mesometrium, rather than to the mother or to an assumed gravitational axis.

*Prelocalization and regulation.*—Experiments to test the developmental capacity of embryonic parts are frequently done by

explantation to culture medium. Mention should, therefore, be made of a report (176) of the cultivation of chick embryo tissues in fully synthetic nutritents. Further tissue culture experiments, (177) with neurons and Schwann cells, reconfirm the principle of "contact guidance" in controlling the direction of outgrowth. It is also shown that tissue fragments give off a colloidal exudate which spreads over the exposed surfaces coating them with a fibrous mat in which the orientation of the fibrils is determined by the direction in which the exudate spreads and in turn determines the direction of outgrowth of the terminal processes of the cells.

In the sea-urchin, appropriate centrifugation of unfertilized eggs (178, 179) produces granule-free, nucleic acid containing (180) fractions which, when fertilized, can develop into normal plutei. Thus the granules are evidently not necessary for normal development, but it is noted that mitochondria and pigment appear as new formations in the plutei. Isolates of the blastomeres of the two-cell to the thirty-two cell stage of *Nereis* eggs are found to differentiate only the structures they would normally have formed (181). Earlier work had shown that "mosaic" eggs of this type, including *Nereis*, possess sufficient regulative capacity to form double monsters as a result of equalization of the first cleavage. To account for the apparent paradox it is suggested (181) that the procedures producing equal cleavage set up effective barriers between different regions of the embryo.

Gastrula ectoderm of *Amblystoma punctatum* frequently forms neural tissue when explanted to salt solution. This, according to new evidence (182), may result from inductive influences released by disintegration of some of the ectoderm cells which, in this species, are especially sensitive to dispersive action of the standard solution. Cultural conditions that prevent this are described. Portions of late gastrula ectoderm of the frog implanted into the tail regeneration blastema of tadpoles differentiate into a variety of tissues almost regardless of the region of origin. However, new explanation experiments (183) in salt solution show that such structures as horny jaws, nasal sacs, and lenses are formed only from ectoderm taken from the vicinity of their presumptive location, although suckers may develop from nearly any part of the ectoderm. The endoderm of tree frog neurulae is found (184) to lack capacity to regulate histologically, but to be capable of doing so morphologically, in experiments on reversed fusion of lateral



half embryos, extirpation, addition, and rotation of parts. The neural crest of salamander neurulae has been the subject of extensive investigations (185 to 189) by vital staining, extirpation and transplantation methods, relating to its origin and fate and particularly to its role, along with that of adjacent medullary plate, in the formation of the chondrocranium and teeth. It now appears well established that the neural crest not only forms the spinal ganglia, the transient ganglion cells of Rohon and Beard, and the pigment cells of the skin and viscera (except perhaps epidermal melanophores), but contributes to the cartilaginous cranium (but not the axial skeleton) and to connective tissue of the head and trunk. Whether or not they form the sheath cells of Schwann seems still to be in dispute (188, 189). Further transplantation and extirpation experiments on amphibian embryos relate to:—the developmental fate of posterior neural plate mesoderm (190), the origin of the blood islands (191) and primordial germ cells (192), the regulative capacity of the heart anlage (193), the totipotency of lateral halves of the mesencephalon at the stage of closed neural folds (194), a demonstration of differential growth rather than migration of cells in the epidermis accompanying growth of underlying structures (195), and an analysis of the orthogonal fibrillar structure of the basement membrane of the epidermis (196). Regulative capacity is also exhibited by the hindgut of the chick embryo in the formation of allantoic and caudal intestine (197) and by the imaginal discs of *Drosophila* larvae (198).

*Interactions of tissues and induction.*—Tests (199) of the inductive capacity of medial and of lateral pieces of the archenteric roof show that the former have greater action and induce both neural plate and crest, while the latter are weaker and induce only neural crest. The results are interpreted as supporting Dalcq's view that differences in the specific response of the ectoderm is dependent upon quantitative differences in a single "evocator" substance rather than that qualitatively different substances are involved. In chemical investigations on the neural inductor it is found (200) that protein extracts of neural plate plus chordamesoderm are effective, no differences in the structure of neural tube being evident in extracts of anterior as compared with posterior regions. With frozen dried material, posterior regions induce with the greater frequency. An important role in induction is attributed



(201, 148) to the pentosenucleoproteins on the basis of such features as:—the correlation between ribonucleic acid content and inductive power of different kinds of implants, decrease in inductive ability after ribonuclease treatment, or ribonucleic acid-destroying heat treatment, decrease in ribonucleic acid content in chordamesoderm as it invaginates along with increase in the overlying ectoderm.

Experiments (202) on induction of the labyrinth from indifferent ectoderm in xenoplastic transplants between toad and salamander have reaffirmed Spemann's principle that the structure formed is dependent upon location in the host but the specific characteristics reflect the donor; thus, in the present case toad belly ectoderm forms a labyrinth of its own type in the salamander. The histological and functional differentiation of the hypophysis is found (203) to depend upon its contact with the infundibulum, in experiments involving rotation of portions of the presumptive medullary plate of *Hyla* embryos through 180°. In experiments (204) on the effect of peripheral field on the proliferation and differentiation of cells in the central nervous system, the situation in the salamander is found to conform to that in anurans, birds, and mammals, as shown by the increase or decrease in number of proprioceptive neurons of the mesencephalic V nucleus corresponding to changes in size of its field (levator muscles of the jaw).

Incompatibility reactions upon xenoplastic transplantation occur with less frequency in early embryos than in later stages. In a contribution (205) to this subject, transplants of *Triturus* optic cup orthotopically to *Hyla* show complete compatibility in the early embryos for a period of about a week after which degenerative changes occur in the graft. Incompatibility in species chimeras of *Triton alpestris* and *T. palmatus* produced by uniting halves of gastrulae or early neurulae sets in at about metamorphosis and results in death (206). The chief effect is damage in the central nervous system, being strongest in the brain and anterior cord regardless of which species furnishes the head. Effects of age and temperature have been examined in experiments (207) on heteroplastically engrafted eyes in two species of *Amblystoma* in which there is an accelerating action of older hosts on relative growth rate of younger eyes and a retarding action in the reverse situation.

In insects, hormonal influence on imaginal differentiation is shown in experiments (208) on implantation of eye-antenna anlagen into *Drosophila* larvae of suitable ages. Other experiments show the induction of metamorphic changes upon implantation of ring glands into isolated larval abdomen of *Drosophila* (209) and the action of the brain in terminating pupal dormancy in a silkworm (210). In mammals, rapid maturity of embryonic mouse ovaries is induced (211) by transplantation to the ovarian capsule of ovariectomized young adults, and offspring are obtained therefrom. Possible uses of this highly interesting procedure in genetic, hormonal, and pathological studies are suggested (211).

*Action of chemical agents.*—The advances that have been made in biochemistry by the use of specific inhibitors of enzyme systems have encouraged considerable work on effects of such agents in developing embryos. Cyanide is known to permit development of amphibian eggs to proceed up to gastrulation. New tests (212) confirm this and also show that azide stops development immediately at any stage, presumably by interference with some enzyme, such as adenylypyrophosphatase, which might be involved in an anaerobic synthetic process. The development of amphibian eggs can (213) also be reversibly blocked by *p*-nitrophenol, which is of interest in connection with its ability to inhibit assimilatory processes in bacteria (214). A partial dissociation of growth and differentiation is also reported (213) to be effected by dilute solutions of this agent. Another inhibitor, hexenolactone, is found (215) to have differential effects on haploid and diploid frog embryos. There is a report (216) of an acceleration of development in sea-urchins by means of sodium thiocyanate, sodium iodide or methylene blue, but no quantitative data are given. Thiourea reversibly blocks development at the gastrula stage in sea-urchins (217) and, in frogs (218), it inhibits metamorphosis while allowing growth to continue. Thyroxine overcomes the latter effect and it is concluded (218) that the thiourea acts here, as in mammals, by inhibiting the formation of the normal thyroid hormone.

Lithium chloride, as is well known, produces specific morphogenetic effects such as exogastrulation (resulting from endodermization of presumptive ectoderm) in sea-urchins and cyclopia in amphibia. These and related effects of lithium and other agents are the subject of further investigation in echinoids (219, 220) pond snails (221), nereid worms (222), cyclostomes (223),

and amphibians (224 to 232). The various agents produce, in these different groups of animals, diverse types of morphogenetic effects which do not, as yet, appear to be reducible to a common factor; nor can lithium be regarded as occupying a unique position, since other agents can duplicate its effects. These endodermizing agents are now considered (219, 228) to produce their effects as a result of precipitative action on the protoplasmic colloids rather than by inhibition of carbohydrate metabolism as formerly held. An ectodermizing agent, such as thiocyanate, may be partially neutralized by lithium (220) but usually a combination of both effects result. Of special interest is the action of thiocyanate and pyocyanine in producing a hyperdevelopment of the notochord with hyperinduction of neural plate in whole amphibian embryos and a neuralization of ventral gastrular explants (224 to 229, cf. 182). Likewise of interest is the production by lithium of hypomorphic amphibian embryos which, in extreme cases remain in a condition of three undifferentiated germ layers (231, 232).

Recent teratological studies of the action of chemical agents on chick embryos include:—rumplessness induced by insulin (233, 234); strophosomy, polydactylism and celosomy by colchicine (235 to 239); achondroplasia, brachymelia and spina bifida by sulphamides, eserine and other agents (239 to 242). In the fish *Fundulus* treatment of the eggs with magnesium chloride resulted (243) in the formation of a pair of twins that lay in the same meridian of the egg in head to tail position.

Early administration of androgens in opossums is found (244, 245) to induce prostate development and male type urinogenital sinus in females. Prospective potency for prostate development is lost at thirty days after birth. The phallus in either sex differentiates into a male type of structure in response to androgens and female in response to estrogens (246,) and the hormones are shown (245, 246) to act specifically on various histological components, not on the organ as a whole.

*Action of physical agents.*—Effects of centrifugation have been followed in developing eggs of the silkworm (247), the pond snail (248), and the frog (249). The results of the experiments on the snail dispute earlier views of progressive cytoplasmic differentiation between maturation and first cleavage. In the frog, upon axial centrifugation of the uncleaved egg, inhibition of gastrulation and

of organogenesis occurs, the latter even in cases when the former is normal. This is interpreted (249) on the basis of a dislocation of various localized materials whose interaction is necessary for normal development. The action of gravity has also been examined (250, 251) by inversion of the uncleaved egg of the frog. Among the various types of abnormal embryos that result are those described as hyperdorsal and hyperventral, the origin of which evidently depends upon the size of the yolk mass involved in gastrulation.

Further studies (252, 253) on elimination of the germ cells in *Drosophila* by ultraviolet irradiation of the posterior pole of the egg show that the mesodermal portions of the agametic gonads develop normally. There are, however, fewer ovarioles in the agametic ovaries and this would imply a stimulating action of the germ cells on egg-tube formation. In the clothes moth, experiments with localized ultraviolet irradiation have demonstrated (254) the presence of specific imaginal Anlagen in the egg. New x-ray experiments on *Drosophila* carrying homoeotic genes (255) tend to support Waddington's view that diffusible morphogenetic substances released by the dead cells account for the production of phenocopies. Temporary lowering of the body temperature of laying hens is found (256) to result in the production of many twin embryos.

Imbedding amphibian embryos in agar effects (257) a dissociation of the processes of differentiation and expansive growth, and it is suggested on the basis of the experiments that internal hydrostatic pressure is one of the fundamental morphogenetic factors. Data on the breaking strain of cells are presented in a paper (258) dealing with the physical forces that may be of importance in morphogenesis.

*Development of antigens and enzymes.*—In studies on the chick it is reported (259) that antigens having adult organ specificity do not appear in the embryo until the organs are well differentiated morphologically. Thus adult antigenicity for eye lens is first shown at 146 hours incubation; erythrocytes at 100 hours; kidney at 220 hours; brain, testis, and ovary at 260 hours. In early embryonic lens there is also indication of the presence of an antigen that is lost in the adult. On the basis of a few tests with frogs it is stated (259) that the embryonic brain lacked an antigen specific to adult brain. The problem of the development of anti-

gens has been further studied (260) in the frog by use of absorption technique. Antisera prepared against adult brain extract cross-react with serum and extracts of various adult organs, eggs, embryos, and larvae. Upon absorption with frog serum weaker cross-reactions are obtained with extracts of the organs but are much reduced, or totally lacking, with extracts of eggs and embryonic stages prior to blood formation. It is concluded (260) that eggs and early embryos possess antigens or combining groups very similar to those present in the frog serum.

In addition to the work to which reference is made under the heading Embryonic Metabolism, succinic dehydrogenase and succinoxidase have been followed (261) in the cerebral cortex of the fetal pig. The former shows in early stages about one-third the adult activity per unit dry weight of tissue and increases during two critical periods previously described for the cytological differentiation of the fetal cortex. The latter is practically absent in stages up to about 66 days but at birth is equal to that of the adult. The low activity in the early stages is ascribed to low activity of cytochrome.

*Regeneration.*—The presence of a lens of *Amblystoma* (which cannot regenerate a lens from the dorsal rim of the iris) is found (262) to have the same inhibiting action on the iris of *Triturus* (which has that capacity) as does the host's own lens, while lens tissue of *Rana* and wax spheres of lens size show no such inhibiting action. Regression rates in denervated amputated limbs of urodele larvae show (263) temperature characteristics that are similar to those obtained in various growth and development processes. The regression effect is considered (263) to be related to processes of regeneration, since the first phase resembles that phase of regeneration that leads to the formation of the blastema. In *Tubularia* effects of oxygen tension, pH and other agents on rates of regeneration and respiration have been studied (264 to 266). Increasing the oxygen tension increases (264) the oxygen consumption, size of regenerant, and rate of regeneration. The increase in size (length) is explained as due to a proximal shift in threshold of inhibition so that more of the inhibited region adjacent to the base of the regenerant participates in regeneration. It is also shown that the parts of the hydranth are not completely determined until about the last quarter of the regeneration period.

*Functions of larval structure.*—The presence of the notochord is

shown (267) to be important for the elongation of the amphibian tadpole and for its ability to move in response to stimuli. In experiments in *Amblystoma* (268) on the replacement of embryonic midbrain with spinal cord, the early larvae are found to be capable of normal locomotor responses which in later stages are greatly reduced. The swimming mechanism is evidently spinal and autonomous in the early stages and comes under mesencephalic control with the development of the tecto-bulbar and tecto-spinal tracts. In grafting operations on *Fundulus grastrulae*, causing abnormalities in Mauthner's neurons, it is found (269) that asymmetry in arrangement of the fibers is not reflected in asymmetry of swimming reaction, indicating a high degree of regulability of function in the developing brain of this animal. Evidence for bipolar functioning of cells of the intestinal epithelium of the frog tadpole is presented (270) in observations on the elimination of pigment and of injected dyes. Since zones of absorption seem to alternate with zones of elimination, the same cells evidently do not have both functions simultaneously.

#### GENES AND DEVELOPMENT

*Changes in chromosome number.*—The most extensive recent work along this line has been done in salamanders by means of extirpation of the egg nucleus for haploids (271) and cold or heat treatment for polyploids (272 to 276), and includes a comprehensive review (276). Among the many features of special interest is the demonstration (277) that, while cell size is directly proportional to number of chromosome groups, and body size is equal to that in diploids, normal structure of the tissues is maintained by compensatory changes in cell number and shape.

*Sex.*—In *Amblystoma*, by the technique of implanting a gonad preprimordium on one side of the embryo, several adult sexually-reversed individuals that proved capable of reproduction have been obtained (277). Analysis of the progeny provide strong evidence that sex-determination is of the female-heterozygous (WZ, ZZ) type in this species. Among the progeny there occur females that are evidently of the genotype WW. The corresponding YY type does not usually survive in other forms, like *Drosophila*, and it is suggested (277) that the W chromosome here is identical with the Z except for the absence of a male-determining gene or genes. Other studies (273, 276) on haploids and polyploids also

point towards female heterogamety in salamanders. In Hymenoptera sex-determination and control of sex are the subject of excellent reviews (278, 279). It now seems clear, at least in *Habrobracon*, that sex-determination is effected by a series of multiple alleles, of which any combination of two different ones will result in females.

*Pigment patterns.*—Investigation has continued, by means of transplantation and explantation, on the nature of the factors determining the position of the pigment bands in species of *Triturus* (280). While the previous interpretation, based on a "negative affinity" of the melanophores, seems applicable to forms like *T. rivularis* which has a widely dispersed distribution of larval chromatophores, it is less so in forms like *T. torosus* in which all of the melanophores are aggregated into a pair of compact dorsal bands. The present work shows that the dorsal bands results from a secondary reaggregation, at time of melanin formation, following a primary phase of uniform, wide distribution. The genetic setup in *T. rivularis* is evidently such as to prevent the melanophores from attaining the level of differentiation necessary for reaggregation. In birds further striking evidence that pigment pattern is determined largely by the genetic constitution of the melanoblasts themselves is given in experiments (281) involving double transplantation between nonpigmented (White Leghorn) and pigmented (e.g. Barred Plymouth Rock) breeds. Other studies (292) show hormonal influences in the expression of silver in the Silver Campine fowl in which a single sex-linked gene, S, is primarily responsible for the inhibition of development of red melanophores. Castration results in the production of red pigment but injection of male or female sex hormones into the capons suppresses this. From chemical studies (283) the red melanins and melanophores are not considered to be transitional stages in the formation of the black and it is suggested (282) that the beginning of pigment production itself is the critical event in fixing the melanophore as red or black.

*Embryonic effects of genes.*—Tracing the action of genes back to the first stages in which their effects become manifest continues to be a subject of extensive investigation. In mice the manifold defects produced in the axial skeleton and urogenital system by a single mutation are in part evidently (284) secondary to cell degeneration processes in the tail which is the first structure to



show abnormalities in the early embryo. Among the offspring of mice carrying mutations near the *T*-locus, various types of monsters are found (285) whose origin is attributed to primary disturbances of embryonic processes rather than secondary destruction of originally normal anlagen. In rabbits there is evidence (286) of genes having specific regions of influence in effecting presence or absence of extra vertebrae, the extent of the lumbar region being determined by combinations of a number of them. In chickens of polydactylous stock it appears (287) that supernumerary finger primordia may be formed in the embryo and subsequently lost, the loss occurring more commonly in embryos from heterozygous than homozygous parents. The frequency of survival of homozygous Creeper embryos is found (288) to be increased when they are incubated at reduced temperature during the first day. The limitations of transplantation methods for determining whether specific hereditary features result autonomously from local gene action or from diffusible substances elaborated by the particular genes is discussed (289) on the basis of experiments with the eye anlagen of Creeper embryos. Recessive rumpleness in fowl is shown (290) to result from abnormal formation and subsequent regression of tail elements rather than from degeneration of presumptive tail tissue as in the dominant mutant.

In frogs many species-hybrids die at some specific period in early development. A new study (291) of the cross *R. pipiens* ♀ × *R. sylvatica* ♂ shows that the embryos may survive a week beyond the beginning gastrula without making any developmental progress beyond that stage. There appears to be involved here a failure to produce "evocator" or a lack of competence on the part of the ectoderm about which it would be interesting to learn more. In intraspecific crosses, too, various degrees of abnormality and inviability are obtained and tests (292) with races of *R. pipiens* permits a seriation that corresponds roughly to the latitude of their distribution. Since the "races" are differently adapted in respect to temperature tolerance, rate of development, size of egg, etc., it is suggested (292) that evolution, here, involves the synthesis of adaptive genomes which can incidentally act to isolate the populations in which they occur. Work on lethal deficiencies is the subject of a review (293) that summarizes the evidence for distinct developmental effects of different genes in the X-chromosome of



*Drosophila* and that includes data on oxygen uptake of single eggs, showing that X-deficient eggs start out normally but, at the time of appearance of the visible abnormalities, the rate falls off markedly to a value characteristic of unfertilized eggs. It is suggested that an entire enzyme system is inactivated in the deficient eggs, but evidence is lacking in regard to this. For assessment of relative effects of nucleus and cytoplasm, androgenesis is a useful procedure, but it is limited by the failure of the haploid individuals to reach late stages of development. This difficulty might not be expected to arise in forms like the bees and the wasps which normally produce haploid males; and it has, in fact, now been shown (294) that normal, mature, androgenetic haploids result from the fertilization of x-rayed eggs of *Habrobracon*.

An interesting case of natural transplantation has been reported (295) in a study of the erythrocyte antigens of bovine twins. It appears from this work that, between certain twins with vascular anastomoses, there occurs an interchange of erythroblasts which become established in the hemopoietic tissues and produce erythrocytes bearing antigens distinct from those of the host. That no incompatibility reaction occurs is somewhat surprising but perhaps no more so than in the analogous case (281) of the persistence of foreign melanoblasts in fowl.

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DEPARTMENT OF EMBRYOLOGY  
CALIFORNIA INSTITUTE OF TECHNOLOGY  
PASADENA, CALIFORNIA

## REPRODUCTION<sup>1</sup>

BY G. VAN WAGENEN

*Department of Obstetrics and Gynecology,  
Yale University, New Haven, Connecticut*

This year has seen the welcome reappearance of books and journals with familiar names from abroad. Notable additions to the literature of reproduction were Asdell's *Patterns of Mammalian Reproduction* (1) and Burrows' *Biological Actions of Sex Hormones*. From France came Courrier's *Endocrinologie de la Gestation* (3), Mme. Moricard's *Hormonologie Sexuelle Humaine* (4), and Caullery's *Biologie des Jumeaux* (5), and from the Soviet Union Zavodovsky's *Management of Processes of Reproduction of Animals* (6). Subjects of review articles included: the mating of mammals (7), the copulatory response of small mammals (8), menstruation (9), the hormonal induction of estrus (10), and the metabolism and motility of sperm (11), the last two with numerous references to Russian works. While considerations of endocrine control continue to dominate the field, the role of enzyme systems in certain integral processes is receiving increased attention. The effects of ionizing radiation on the reproductive system have caught the popular interest. Some details are already available (12, 13) and doubtless more information will be forthcoming as a result of recent tests. Necessarily, the subject matter of the present review has had to be limited principally to mammalian reproduction.

*The menstrual cycle.*—Studies on the cycle of the rhesus monkey (*Macaca mulatta*) by several schools now cover two decades or more, and many of its features are well known; the temporal sequence of events, as emphasized by a previous writer in this series (14), continues to hold interest for study. In the current year Corner (15) has described the life history of the corpus luteum in this animal. The theca interna cells of women appeared to contribute significantly to the corpus luteum and to be added to it throughout its functional life (16). On the basis of menses, ovarian picture, age of embryos, and time of ovulation (when known), Bartelmez *et al.*, (17) reconstructed events of the rhesus cycle; these were a phase of postmenstrual activity concerned with endometrial

<sup>1</sup> This review covers the period from July, 1945 to August, 1946.

reorganization, four phases associated with growth and regression of the corpus luteum, and a brief phase of postmenstrual repair. The duration of these phases was as variable as that of the cycle itself.

The mean length of normal cycles of the baboon (*Papio ursinus*) was  $39.6 \pm 0.2$  days (range twenty-nine to forty-two days), divided into two phases of sex skin, turgescence and deturgescence. The first phase, dominated by the estrogenic hormone, was more variable and more easily disturbed experimentally than the second, this being attributed to an in-co-ordination of processes responsible for ovulation and corpus luteum formation (18). Irregular cycles in the baboon were related to the appearance of macroscopic yellow patches in the ovary arising as lutein changes in cells of the theca interna, but the hormonal attributes of these bodies are unknown (19). A possible parallel to the unstable first period of the baboon may be the finding in macaques of four cases of delayed ovulation occurring on the twenty-ninth to the thirty-seventh day, indicating unusually long periods of postmenstrual inactivity (20). The authors suggest that such cases might explain reports of ovulation during the catamenia in women; however, it would seem likely that these animals were physiologically at midcycle. When restricted (forty-eight hour) matings in macaques were deferred to the seventeenth day of the cycle, a single pregnancy resulted from eighty matings in twenty-five animals (the expectancy is 30 per cent with eleventh to thirteenth day matings). The seventeenth day is therefore too late, in relation to ovulation time and ovum viability, for successful impregnation (21). Epithelial changes in the cervical canal and glands of the monkey may provide an index of hormonal changes in the cycle, since mucosal growth and regression curves paralleled blood estrogen curves for normal women (22). Of interest to those concerned with the housing, surgery, and breeding of primates is the report of Kennard, Ruch & Fulton (23).

Timing of ovulation in the human subject by means of basal temperature shifts appears to be related to the lowering of body temperature by estrogen and its raising by progesterone (24, 25). Large doses of chorionic gonadotrophin administered late in the luteal phase in two women maintained the corpora lutea (26). The mechanism was unstated but might well have been an effect of estrogen (cf. 27). A new theory of menstruation was advanced, based on the short-circuiting of blood flow in arteriovenous anasto-

moses newly described in the endometrium; with increased growth of the mucosa, a crisis in oxygen supply and breakdown of the mucosa is initiated (28). An excellently illustrated comparative study of the vaginal smear cycle of the rhesus monkey and the human provides a clear working classification of cell types (29). Evaluations of the use of vaginal smears in diagnosis have appeared by the originator of this method (30) and others (31, 32, 33). In an entertaining article on adolescent sterility in the human female (34) the criticism appears that "puberty, menarche, maturity, and adolescence" are terms that are used all too loosely; to them may be added the term "estrus," as employed in this same article.

*The reproductive cycle in other mammals.*—Information is accumulating rapidly on the cycle of the golden hamster (*Cricetus aureatus*) which, by reason of its short cycles and pregnancy span, early maturity, large litters, and perhaps its novelty, is becoming well established as a laboratory animal (35 to 39). Hormonal factors responsible for estrus were similar to those obtaining in the guinea pig, rat, and mouse. The castrate hamster was relatively less sensitive to estrogen than the other rodents in the elicitation of behavioral estrus, but addition of progesterone regularly provoked this response (40). The length of the cycle of the rat could be manipulated at will by administration of progesterone, estrogen, or both, in diestrus (41). Differing from rodents, the cow achieved full psychic heat with estrogen alone (42) and its threshold was so low that intense manifestations, characteristic of the normal animal, were easily provoked in the castrate cow (43). In those cases where estrogen alone precipitates estrus in spayed animals, participation of progesterone-like steroids arising in the adrenal gland is considered not unlikely (43, 44). Testosterone propionate evoked mating behavior in adult rats that had been castrated as early as the first day of life, the only differences from normal being attributable to impaired development of the penis (45). Certain conclusions were reached regarding the reproductive cycles of the fur seal (46) and the whalebone whale (47).

It is possible to modify the reproductive system during periods of natural quiescence. Graafian follicles in the anestrus opossum gave varying responses to injected follicle stimulating hormone, from partial development to full ovulation (48), while ovariectomy led to further regression of the accessories, pointing to a residual estrogenic control (49). In the opossum, from a study of the re-

sponse to progesterone, Nelson (50) also concluded that estrogen secretion began at the fourth month of life and continued into the anestrus. The ground squirrel (*Citellus tridecemlineatus*), though normally monestrous, could, if the fetus were lost during pregnancy, experience a second estrus to give a "replacement pregnancy" (51). There was a seasonal difference in the responses of the follicles in the bat (*Myotis grisescens*) to pregnancy urine gonadotrophin (52) and of the rabbit ovary to pituitary gonadotrophin (53), the latter phenomenon occurring in an animal not usually considered to exhibit great seasonal variations in fertility. While ovulation has frequently been induced in anestrus ewes, conditions for success are by no means defined (54, 55, 56). The largest percentage of matings and lambs appeared to follow two injections of equine gonadotrophin given at an interval of sixteen days (54). In the lactating sow, estrus is normally inhibited until the sixty-eighth day; using equine gonadotrophin, ovulation, followed by successful breeding, was induced on or after the thirty-eighth day (57).

*Biology of the sex cells.*—Studies of the enzymatic mechanisms involved in the respiration of sperm have continued to hold interest. On an egg-yolk phosphate substrate, oxygen uptake and fertilizing capacity of sperm ran parallel. A dialysable inhibitor was separated from egg yolk, and the residue used successfully in media for sperm storage (58). In the glycolytic cycle, a decrease in adenosinetriphosphate was correlated with loss in motility (59). The endogenous glycolysable sugar of whole semen of bull, boar, and ram (60) and of man (61) proved to be fructose. Assays of semen hyaluronidase were suggested as an index of fertility (62). The postulated function of this enzyme in follicle cell dispersion led to its first use in artificial insemination in man with reported success (63). Hyaluronidase was present in the reproductive tract of the rabbit immediately after coitus, but its synthesis appeared to have ceased since the ratio of enzyme to sperm decreased with time (64).

*Fertilization.*—Up to the fifth day, the nutritional requirements of the mammalian ovum are satisfiable by the "deutoplasmic" substances of the egg itself (65). A decrease in volume of the ovum of the hamster at fertilization and first cleavage was held to provide the energy required for cell division (66). A high incidence (30 per cent) of degenerate eggs found in gonadotrophin-induced ovulation in hypophysectomized rats followed by mating was explained by time relations with respect to the age of the eggs at ovulation, or to

the uncontrolled interval between ovulation and fertilization (67). Parthenogenesis to the morula stage of atretic ova in the rodent ovary was described (68). The ova of chickens prematurely ovulated by pituitrin injections failed to undergo important development (69), but, when ovulated by intravenous injection of cockerel anterior pituitary extract, eggs of less than twenty-two hours prematurity reached advanced embryonic development, or were hatched (70).

*Sperm fertility.*—The relation of the semen to sterility was well explored in a recent symposium edited by Engle (71). Male fertility was assessed by traditional methods (72) and attempts made to standardize techniques for determining density, morphology, motility, and viability (73). However, analyses of semen from forty normal fertile men showed as the only consistent finding a high percentage of morphologically normal forms (74). Even this may not be an absolute criterion, for following cold treatment of the scrota of rabbits, physiological deterioration appeared very rapidly, while morphological signs were delayed for a few hours (75).

*Gametic elements and their modification.*—Migration of primordial cells to sex gland primordia in the human occurs by amoeboid movements and lytic destruction of obstacles; ovogonial multiplication approached completion at the fourteenth week (76). Ionizing radiation from atomic bomb action produced in man sloughing of the germinal epithelium and reduction of the contents of seminiferous tubules to a layer of Sertoli cells which conversely appeared to increase in number. These effects were already apparent on the fourth day. The sperm count dropped immediately. The ovaries were more resistant, but, while primary follicles persisted, developing follicles and newly-formed corpora were absent. A corpus luteum of pregnancy in one case appeared little affected (13). Far-reaching testicular degeneration in rats produced by x-rays or estradiol dipropionate was promptly repaired by male hormone (77). After month-long hypophysectomy, the injection of equine gonadotrophin into rats (78) and testosterone propionate into rats and mice (79) restored complete spermatogenesis. Forty-day-old hypophysectomized rats injected with the interstitial cell stimulating hormone showed tubular maintenance but accessory regression, while testosterone propionate stimulated both structures (80). Thus, while these phenomena are now well documented, their intimate mechanism remains unclear. Recovery of spermatogenesis

genic activity in experimental cryptorchidism occurred if the testes were replaced in the scrotum before 150 days, but after this lapse of time recovery was less satisfactory or nil (81).

The degree of sex reversal producible experimentally varies with the species, the time treatment is initiated, and with the amount and duration of hormone dosage. Masculinizing and feminizing effects were described in rudimentary gonads of amphibia by the use of sex and adrenal-cortical hormones (82). Complete sex reversals were obtained in female *Rana clamitans* by injection of larvae with testosterone propionate for a minimum of ninety-five days (83). Androgens administered prenatally to pregnant hamsters or postnatally to their female young had little effect on the ovaries of the offspring (84).

*Endocrine elements and their modification.*—Morphologically the adult mammalian ovary comprises glandular tissues of varying origins and potentialities (85). As a physiological correlate of this, grafted ovaries of the mouse possessed a life-sustaining adrenal-cortical function, in addition to their demonstrated capacity to produce female and male hormones (86). The interstitial gland of the guinea pig tends to a maximum near estrum and in early pregnancy, and to a minimum in mid-pregnancy, but the cycle was poorly marked (87). In the rat corpus it was suggested that adenosine triphosphatase activity was concerned with lipid metabolism and particularly with the formation and release of progesterone (88).

Irradiation of the testes of mice with x-rays gave a relative increase, but absolute decrease in interstitial tissue (89), while after ionizing radiation the appearance of the interstitial tissue indicated hyperplasia rather than compaction (13). The anomalous hypertrophy of Leydig cells in some aged dogs was shown to be a pretumorous, rather than a senile, change (90). Injection of equine gonadotrophin directly into fetal rats increased the size and number of Leydig cells, but gave no evidence of increased hormone production (91). Whether this failure resided in the target or the end-organ is not clear, since mice from five days of age responded by increases in interstitial tissue and in the weights of seminal vesicles, and by the disappearance of the adrenal X-zone (92).

*The genital tract and its modification.*—Experimental modification of structures of the genital tract by hormonal administration has continued along well-established lines; some lesser known



structures have received attention and new animal species studied. Oral administration of several synthetic estrogens to domestic fowl and turkeys showed stilbestrol to be effective on the oviducts of both birds, but dianisylhexane to be very effective in the chick only (93). Estradiol produced hypertrophy of the Müllerian ducts of newly metamorphosed frogs to give an oviduct-like structure (94), and the rudimentary oviducts of newts likewise hypertrophied following stilbestrol treatment (95). The proportion of ciliated cells in the Fallopian tube of the opossum increased after treatment with equine gonadotrophin, estrogens, and androgens, and the first two hormones also stimulated the formation of mucigen granules in nonciliated cells (96). Local injection of estrogens in the vaginal region of immature rats (97) and guinea pigs (98) provides an exceedingly sensitive test for these substances. Estrogen transformed the urethral epithelium of male and female rats to a stratified squamous type (99).

The interactions of estrogens, natural and synthetic, with progesterone, may be unpredictable. Thus, estradiol synergizes progesterone in its action on the rabbit uterine mucosa, a property not shared by estrone or stilbestrol (100). Estrogen is antagonized by 1,500 parts of progesterone (101). With lower relative amounts of progesterone, mucification of the vagina may occur (102), but absolute hormone levels as well as their ratio was shown to be important in determining the occurrence of synergism or suppression (103). Progesterone inhibited the action of estrone on the chick oviduct; the response was proportional to the dose up to a maximum, at which point even a sixteen-fold dose of estrogen was unable to overcome the inhibition (104). The sensitivity of the Müllerian system of the opossum to androgens was confirmed (105, 106). The response of the seminal vesicles of the rat to testosterone propionate was made the basis of an assay method (107). Prostate and seminal vesicle responses to injected testosterone propionate began to show regression after two weeks in normal but not in castrate rats (108), indicating gonadal suppression. In the castrate mouse, from the appearance of the accessories, the adrenal gland did not produce appreciable amounts of androgen (109).

*Pituitary-gonad relationship.*—Distribution of cell types in the anterior pituitary of the rat in late pregnancy and lactation suggested the acidophils as the source of lactogen (110), and a similar conclusion was reached for the specific azocarmine-staining acido-



phils of the cat (111). A strong indication that lactogenic hormone and luteotrophin are identical (112) was further supported (113). The hypophyses of castrate male rats were larger, and those of females smaller, than controls. Basophils showed hypertrophy, evidence of some increased function, and some degeneration (114). Curious anomalous effects (permanent estrus, with castrate type pituitary) followed ligation of the ovarian pedicle; administered estrogens corrected the latter effect, and hypophysectomy abolished estrogen hyperproduction and cyst formation (115). Castration cells in the hypophysis of man were described following destruction of the gametic elements by gamma rays and neutrons (13). Enlargement of the pars nervosa of the hamster following chronic treatment with diethylstilbestrol was confirmed (116) with the exception that no invasion of the brain tissue of the tuber was seen. The pathway for the ovulatory mechanism of the rabbit came again under review, with the finding that direct electrical stimulation of the hypophysis caused ovulation only when the current irradiated to the hypothalamus. It was suggested that the hypophysis is activated humorally via the hypothal-hypophyseal system (117). The Biskind technique of intrasplenic ovarian grafts applied to the castrate guinea pig permitted the effects of physiologically castrate hypophyses to be studied *in situ*. The grafts showed blood follicles at first, and later very extensive luteinization, interpreted as antagonistic intraovarian effects of different hypophyseal gonadotrophins (118). The final picture resembled the theca cell tumors of the ovaries of women.

Castration two to three hours after the injection of equine gonadotrophin prevented, but later castration permitted, the estrous response (119). Using the method of antihormone injection, Zondek (120) found that pregnancy urine preparations required the presence of the ovary for twenty-four to thirty hours. This faster action of equine gonadotrophin was also demonstrated in the immature rat (121).

*Miscellaneous observations.*—After hypophysectomy in domestic pigs gonads and accessories remain immature, and growth is inhibited, but obesity is frequently unchecked (122). Proof that antigonadotrophins act extra-hypophyseally seems complete (123). Contributory to clinical studies is a method of concentrating urinary gonadotrophins by ultrafiltration (124).

*Pregnancy.*—Following atomic bomb action numerous abor-

tions and miscarriages occurred and their incidence appeared to be directly related to nearness to ground zero (13). However, in such a complex situation, it was found difficult to evaluate the factors other than radiation which might have contributed to these effects of mass radiation upon reproduction. The nonessentiality of the hypophysis for maintenance of pregnancy in the monkey was conclusively established (125). In animals operated upon from the 32nd to the 106th day, pregnancy persisted for 131 to 50 days and parturition occurred at the 159th to 186th days. Labor was prolonged and three uterotomies for removal of the infants in a total of six animals was necessary. Lactation, as would be expected, failed. Fetal death occurred in the rat in the absence of the thyroid (126) and the adrenal (127). Following the latter operation, sodium chloride administration prevented fetal death, while desoxycorticosterone prolonged parturition and led to excessive growth of the fetuses and difficulty in delivery. Animals that produce uniovular litters (primates, edentates, some ungulates and rodents, and the bats) have no phylogenetic or taxonomic affinities, but possess in common the reduction of the mammary glands to a single functioning pair, diminution of the uterine cornua with enlargement of the corpus uteri, and reduction to a minimum in the number of ova shed (128). Descriptions were given of the first case of monozygotic twins in the dog (129) and of a quadriovular quadruplet pregnancy in man (130). Socio-statistical data on twinning was provided by Strandskov (131, 132).

*Relations of the placenta.*—In the colyledonary placenta of the sheep, blood flows in opposite directions through parallel nets of maternal and fetal capillaries (133). Placentation in a baboon (26½ day, 5.2 mm. embryo) resembled that in the macaque, the main differences being the presence of an incomplete decidua capsularis and the absence of an accessory placenta (134). Estrone, bilateral ovariectomy, and equine gonadotrophin caused decidual necrosis and fetal death in the rat provided that they acted before the tenth or eleventh day; the placentas differentiated and survived, as in earlier experiments (135). Maintenance of pregnancy in the primate, also, does not depend on the presence of the fetus (136). While these findings, together with that of Smith (125) on the dispensability of the hypophysis, point to the dominance of the placenta in pregnancy, the factors precipitating labor are still unknown. In the human placenta, nuclear volume as a percentage of

cytoplasmic volume decreased steadily from two months to term, indicating a process of uniform ageing, and there was no correlation between placental senescence and the onset of labor (137).

Continuing their studies on histochemical reactions in the placentas of several forms, Wislocki & Dempsey described the distribution and significance of placental glycogen, iron, calcium, lipoids, basophilia, and alkaline and acid phosphatases in the mouse, rat, guinea pig, rabbit, and hamster (138), the cat (139), the pig (140), and the human (141, 142). Similarly, localization of alkaline phosphatase in the placenta and fetal membranes of the guinea pig from eleven days to term is minutely described (143). For the wealth of detail and interpretations, the original papers must be consulted.

*The uterus.*—Embryos became oriented correctly with respect to an experimentally rotated segment of uterine horn in the rat, but not to the mother or to a gravitational axis (144). In intra-ocular transplants of endometrium from the monkey certain combinations of estrogen and progesterone reproduced normal changes in the coiled arteries (145). The metrial gland in the mouse failed to ingest or to prevent passage of trypan blue to the fetus, apparently ruling out a role in phagocytosis (146). Pregnancy in the rat was marked by increasingly abundant uterine epithelial fat (147) and by an increase in uterine elastica, which persisted as tangled clumps long after parturition (148). Following pregnancy the uterine arteries of elephantulus became invested with deposits of collagen and elastica, a phenomenon described as a reversible sclerosis (149). A theory of constant production and absorption of amniotic fluid was advanced (150).

*Hormones in pregnancy.*—In the mouse an early picture of high progesterone and low estrogen was reversed at midpregnancy (151). From the urine of pregnant women was obtained adrenocorticotrophic hormone, not yet identified with the pituitary principle (152). Relaxin was concentrated from pregnancy blood and urine (153) and it is to be hoped that the identity of this elusive hormone will be cleared up. Evidence for an increased antidiuretic hormone production in pregnancy, or for its connection with eclampsia proved unconvincing (154). Antihormones to the pregnancy gonadotrophins are not formed and can not be invoked to explain the amenorrhea of pregnancy (155). The substance in late-

pregnancy mare serum causing inhibition in mares and test animals is apparently estrogen (156).

A number of studies have sought to determine whether in certain abnormal pregnancy states the hormonal balance has deviated from the normal. Negative pregnandiol and positive Friedman tests were prognostic of abortion (157). In pregnancy with co-existing diabetes, toxemias were variously associated with low pregnandiol and estriol (158), progesterone and estrogen withdrawal (159), and low pregnandiol with a rise in chorionic gonadotrophin (160). In early pregnancy a fall in pregnandiol with high estrogen was indicative of abortion of endocrine origin (161). While low progesterone is the common finding, the causal relation of the other hormones to the maintenance of pregnancy remains to be clarified.

*Pregnancy tests.*—Two thousand consecutive routine tests, using the Aschheim-Zondek reaction, gave 4 errors in 649 pregnancies and 5 errors in 174 nonpregnant cases (162). Pregnanediol excretion may be high enough in the second half of the cycle to give a positive Guterman reaction (163). However, using certain precautions, the test was 92 per cent accurate in 248 pregnancies (164), and it seems to agree well with the Friedman reaction (165). After the fortieth day, the Hogben test, using the South African horned toad, was 96 per cent accurate (166). Speedier tests, using the rat, rely on the production of ovarian hyperemia in two hours (167, 168), or six hours (169), and on ovulation in the mouse in eighteen hours (170).

*Placental barrier.*—Administered penicillin and streptomycin appeared rapidly in human fetal blood and amniotic fluid (171, 172). Alloxan reached the fetal circulation of the rat  $1\frac{1}{2}$  minutes after dosage of the mother; it failed to cause permanent diabetes in the offspring (173). Thiourea in the same animal evoked hyperplastic thyroids in the fetuses (174). Adrenotrophic hormone and even desoxycorticosterone appeared to be retained by the placental barrier, if absence of effects in the fetus be accepted as a criterion (127). A method for sex prognosis, using the blood or urine of the mother, suggests a reciprocal exchange of substances about whose nature we are regrettably in the dark (175).

*Mammary glands, lactation, milk.*—Lactogenic hormone administered to puerperal unsuckled mice maintained the secretory

level for five days longer than controls, and retarded parenchymal involution (176). Evidence of the influence of other glands of internal secretion on lactation continues to accrue. Adrenalectomy caused scarcity of milk (127) and parathyroidectomy a marked decline (177). Thyroxin caused a substantial increase in the milk yield of the cow, combined with a negative nitrogen balance (178).

Milk yield in heifers following stilbestrol or hexestrol implants approached that of normal calving (179). In lactating women stilbestrol effectively decreased lactation (180) and it must be accepted that the cause of these apparently contradictory actions is unresolved. To cite a parallel dilemma, orchiectomy was followed by remarkable improvement in carcinoma of the male breast (181), while large amounts of testosterone propionate also exerted a favorable influence (182).

Lactation in the mouse was marked by absence of estrogen and high levels of progesterone (183). Testosterone propionate administered to weaned female rats induced alveolar growth, but when given to mothers, the mammae of the young developed and lactated (184). In the rat, relaxin has been linked to mammary gland growth (185). Several features of milk secretion, including its biochemistry and energetics, were discussed (186) and a complete study of human milk was initiated by Macy and co-workers (187).

*Nutritional aspects of reproduction.*—The literature of prenatal deficiency has been effectively brought up to date (188). There is a highly significant relationship in disabilities of both mother and offspring to the maternal dietary (189, 190). Vitamin E deficient monkeys showed progressive muscular weakness for several weeks prior to death or sacrifice, with pigmentary changes in smooth muscle generally (191). Studies on reproductive behavior of the primate in vitamin E deficiency will be awaited with interest, especially in view of the finding that plasma levels of the vitamin rise during pregnancy (192). The macroscopic uterine pigmentation of rats on an E-free diet is due to a "lipofuscin" deposited in the cytoplasm of muscle fibers and the irreversible sterility after ten months to extensive myometrial fibrosis (193). Vitamin A reserves of the calf fetus were increased by the administration to the mother of a million USP units daily; it was noted that low vitamin A in the fetus might suffice in intra-uterine life, but not later (194). Sterility was induced in growing rats on a tryptophane deficient

diet (195) and the possible connection of sex hormones with dietary factors was elsewhere emphasized (196). To some extent the gonads are spared in acute starvation (197).

Diets adequate for normal and pregnant dogs were inadequate for lactating animals and the nursing deficiencies were corrected by the addition of folic acid to the diet of the puppies (198). In wartime England a 20 per cent incidence of glossitis and heartburn in 900 pregnancies was imputed to riboflavin deficiency (199). While the necessity for an adequate protein nutrition in pregnancy scarcely admits of doubt, actual establishment of standards awaits rigorous experimentation.

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DEPARTMENT OF OBSTETRICS AND GYNECOLOGY  
YALE UNIVERSITY  
NEW HAVEN, CONNECTICUT

## METABOLIC FUNCTIONS OF THE ENDOCRINE GLANDS<sup>1</sup>

By F. D. W. LUKENS

*The George S. Cox Medical Research Institute, University  
of Pennsylvania, Philadelphia, Pennsylvania*

It is hoped that the major fields of work are represented in this review, although Shakespeare's appeal "Piece out our imperfections with your thoughts" must be humbly repeated. Investigators in these fields have the combination of background and criticism required to assimilate such an array of facts. The inexperienced reader should be warned that the time-partition of this type of review tends to separate current facts from the body of knowledge which grows slowly through the years.

### THE THYROID AND THYROTROPIC HORMONES

Most of the contributions to thyroid physiology in the past year have stemmed from the study of the antithyroid drugs and the subject has been recently reviewed (1, 2). In many metabolic experiments the methods permit one to regard the action of thyroxine, thyroglobulin, and thyroid extract as the same. However, the presence of an unidentified form of thyroid hormone other than pure thyroxine or thyroglobulin in the blood of hyperthyroid animals has been postulated (3) since hyperthyroid serum induced a prompt elevation of the metabolic rate in surviving tissues from normal animals, whereas thyroxine added to normal blood failed to do so. The recent symposium on iodinated proteins is also noted (4 to 12).

Iodine deficiency and colloid goiter have received no direct attention, nor has any information accrued on the question of why the iodine treatment which benefits Graves' disease fails to cause hypothyroidism in the normal animal. The chemical conversion of diiodotyrosine to thyroxine was accelerated by iodine and hydrogen peroxide, profoundly influenced by pH, and prevented by the exclusion of atmospheric oxygen and by thiosulfate (13, 14). Experimental evidence has been obtained to support the concept that the agent effective in bringing about the oxidation of diiodotyrosine

<sup>1</sup> This review covers the period from June, 1945 to June, 1946.

to thyroxine is free iodine (13, 8). The rapid transformation of iodine to diiodotyrosine in the study of goitrous thyroids with radioactive iodine was also observed (15). The kinetics of the formation of diiodohistidine resembles that seen in the formation of diiodotyrosine (16). Iodine reacts only with the tyrosine residues in serum albumin and urea accelerates the rate of formation of diiodotyrosine (17). The catalytic effects of phosphate and acetate buffers on the iodination of tyrosine were also studied (18). In glands activated by cold or by thyrotropic hormone the oxidation-reduction potential of the colloid was observed to rise to the same level as in the cells. (19). Thiourea acted *in vitro* and *in vivo*, lowering the potential of cells and colloid of activated glands. However, sulfanilamide, another thyroid inhibitor, did not act by changing the oxygen-reduction potential. Miller, Hoblin & Astwood (20) found that the oxidation of 2-thiouracil by iodine occurred at such a rate that tyrosine and casein were protected against iodination by this compound. They note that this reaction with iodine is not the only factor to be considered, as some compounds inactive on the thyroid are equally reactive toward iodine.

"These results indicate that the reaction with iodine is a plausible explanation for the action of thiourea type antithyroid compounds. These compounds may also decrease iodine liberation by action on the appropriate oxidative enzymes. Thiourea and sulfanilamide, representatives of two types of thyroid compounds, do inhibit peroxidase action and the activity of the second type might be explained by such an effect."

The oxidase system inhibited by thiouracil is not the cytochrome-cytochrome oxidase system of thyroid slices (21, 22), although this system was known from previous work to be concerned with the synthesis of diiodotyrosine and thyroxine. The sulfonamides did not inhibit the succinoxidase system (22). Although the oxidizing systems are not fully understood, these current studies are in fair agreement. By a series of oxidation reactions iodide is converted to iodine which combines with tyrosine to form diiodotyrosine. Diiodotyrosine in the presence of iodine and oxygen then forms thyroxine, which may be stored or secreted as thyroglobulin or in some unidentified form. This system is under the control of hormones. The thyrotropic hormone stimulates the secretion of thyroid hormone and is in turn inhibited by thyroid hormone. The homeostasis of this thyroid-pituitary axis is influenced by other factors such as the supply of iodine, cold, and other stresses.

*Thyrotropic hormone.*—A simplified method of purifying thyrotropic hormone has been outlined by Ciereszko (23). Waldo & Dempsey (24) have indicated that the secretion of thyrotropic hormone was not influenced by water intake. The effect of this pituitary hormone on oxidation-reduction potentials has been noted (19). In addition, De Robertis & Grasso (25) have found that in thyroid glands activated by cold or by thyrotropic hormone, the peroxidase reaction becomes positive in the colloid. Their histochemical methods confirmed the finding of oxidases by Dempsey (26). They answered the chemical objections of Glock (27) by the use of exsanguinated animals, and by distinguishing pure peroxidase from hemoglobin effects by heat treatment of the sections. Astwood (1) states that, like Glock, he has been unable to isolate a peroxidase by chemical means. A final answer must await further work. Barker (28) observed that thyrotropin, which caused 32 per cent increase in metabolic rate in normal rats, caused only 7 per cent increase in thiouracil-treated rats. This further supports the concept that the action of thiouracil is principally exerted on the production of thyroid hormone. Rawson *et al.* (29) recall the earlier observation (30) that thyroid tissue inactivates thyrotropic hormone *in vitro*. They refer to their unpublished studies in which this inactivation was almost completely inhibited if iodine was contained in the medium. The reduction of the proteolytic activity of the thyroid by iodine treatment may be related to this, although De Robertis & Nowinski (31) regarded the proteolytic enzymes as being directly concerned in the release of colloid. Dobyns (32) correlated the changes in the orbit and other tissues with the production of exophthalmos by thyrotropic hormone.

*Thyroxin.*—The determination of plasma iodine and of thyroxin in the thyroid gland has been studied by Taurog & Chaikoff (33, 34) and radioactive thyroxin has been prepared (35). Tipton *et al.* (36) found that the activities of succinoxidase and cytochrome oxidase increased in the liver tissue of rats fed desiccated thyroid. The increase was less marked if the hyperthyroid rats were adrenalectomized. They point out that this is probably a part of the general increase in metabolism and not necessarily a primary action of the hormone. In liver and kidney slices of normal and thyroidectomized rats treated with thyroxin a fall in oxygen uptake occurs after thyroidectomy and both tissues return to their respective "thyrotoxic" levels after thyroxin (37). The observations reveal

differences in the response of the liver and kidney slices to these procedures. Thyroxin activity in rats is greatly intensified in the absence of the liver (38). The liver deals with excessive hormone by some process of inactivation and not by simple excretion. On the other hand, euthyroid subjects have a greater tolerance than myxedematous patients for thyroid extract leading to the conclusion that the normal thyroid gland inactivates the excess thyroid hormone (39). The effect of thyroxin on the nitrogen and calcium metabolism of the lactating cow has been studied (40). Thyroxin did not inhibit the action of estrogen on endosteal bone formation or on the growth of the oviduct, but it prevented the increases in plasma fat, calcium and phosphorus induced by estrogen (41). Danowski, Man & Winkler (42) studied the response of normal and thyroidectomized dogs to thiouracil and thyroxin and pointed out striking differences between the dog and man which indicate that the metabolic processes of the dog are less dependent than are those of man upon the hormones produced by the thyroid. Astwood (1) cites other studies on species differences. Hughes (43) has re-examined the duration of action of single doses of thyroid powder and thyroxin. In rats pretreated with thiouracil the expected hypertrophy of the thyroid was delayed for the duration of action of the test dose of thyroxin. The duration of action of any given dosage was somewhat shorter than by older methods of determining this. Parkes (44) examined the relation between iodine content and biological activity of thyroid preparations and confirmed the opinion that the acid-insoluble iodine of thyroid preparations is all thyroxin iodine. Winkler, Riggs & Mann (45) by observing the relationship between thyroid dose and serum iodine levels in hypothyroid patients, estimated that the daily production of hormone by the normally functioning thyroid glands was approximately equivalent to three grains of U.S.P. dessicated thyroid. Serum iodine levels responded more rapidly than changes in metabolic rate to alterations in thyroid status.

*Antithyroid substances.*—The relation of chemical structure to antithyroid function has been extended by numerous reports (46 to 50). The determination of the more active compounds in serum has been described by Christensen (51). Promizole has been studied by Higgins (52). Astwood & Vanderlaan (53) have reported the initial use of propylthiouracil in man. This type of substance was about five times as active as thiouracil and was preferred by them in the treatment of hyperthyroidism.



*Anatomical effects of thiouracil.*—The changes induced by anti-thyroid agents on the thyroid gland have received continued attention. Thiouracil is transmitted through the egg of the domestic fowl to the chick (54). Treatment of thyroid disease preoperatively with thiouracil indicates that the latter prevents the production of new colloid but does not inhibit the utilization of available colloid in the thyroid gland (55). Thomas (56) compared the vascular bed of normal and thiourea activated thyroid glands in rats. Paschkis *et al.* (57) noted the increased mitotic activity accompanying the hyperplasia of the thyroid and call attention to the possibility of inducing malignancy in goiters exposed to thiouracil. By combined treatment with 2-acetyl amino fluorine and allyl-thiourea, Bielschowsky (58) produced nodular thyroids in rats. Hyperthyroidism due to metastatic carcinoma of the thyroid was completely controlled by thiouracil although the malignancy was not influenced (59). During treatment the excretion of radioactive iodine was increased, a result supporting the conclusion (55) that this drug does not prevent the utilization of available colloid. Thiouracil partially protected rats against the loss of weight from restricted water intake (24). Leatham (60) found that the ratios of organ weight to body weight were unchanged in rats which lost body weight from the reduced food intake induced by thiourea. These results exclude the thyroid which was hyperplastic in the treated rats. He also noted that the gonadotropic hormone content of the pituitaries of thiourea-treated and pair fed control rats did not differ. The administration of thiourea (61) did not affect the distribution of neutral fat and cholesterol in the livers or bodies of rats, although the weight and glycogen content of the liver were increased. In a necropsy on a patient whose thyrotoxicosis was being controlled by thiouracil, Reveno (62) reported hyperplasia and colloid retention of the thyroid, increased basophilia in the pituitary and hyperplasia of the bone marrow and splenic pulp. Vogel & McGavack (63) followed the blood picture in rats given thiouracil. There was mild anemia without progressive leucopenia. Necropsy findings conformed to those of others, eight rats dying apparently of renal injury.

*Toxicity of antithyroid compounds.*—Marine & Baumann (64) reported that thiourea intoxication in rats is influenced by age and sex. Thyroid feeding increased and thyroidectomy decreased the susceptibility to intoxication. Adrenalectomy did not increase the toxicity of thiourea. Higgins (52) included data on the blood pic-



ture of rats given promizole. Daft *et al.* (65) found that thyroxin increased the incidence of leucopenia in rats given thouraea and that the leucopenia was corrected by treatment with *L. casei* factor. Fishberg & Vorzimer (66) state that the natural recovery from leucopenia in patients treated with thiouracil was accelerated by pyridoxine, but Warren (67) found that pyridoxine did not correct the depressed respiration of bone marrow and leucocytes *in vitro* which was induced by thiouracil. Williams *et al.* (68) analyzed the toxic effects of thiouracil in 247 patients, and Moore (69) compiled a cooperative study of thiouracil toxicity in 1,091 patients from many hospitals. In spite of the development of neutropenia, thiouracil did not benefit myeloid leukemia (70).

Thiouracil lowers the incidence of dietary cirrhosis of the liver in rats (71), increases the resistance to reduced barometric pressure (72), interferes with the estrous cycle and lowers the heart rate (74), and diminishes cardiac response to epinephrine in patients (75). Thiourea induces suprarenal cortical deficiency and a decreased liver glycogen (73) and interferes with the development of sea urchin eggs (76).

Numerous reports have appeared on the use of antithyroid drugs in thyrotoxicosis (77 to 83). In addition Chapman & Evans (84) and Hertz & Roberts (85) have reviewed the treatment of hyperthyroidism with radioactive iodine. Discussion of hyperthyroidism in man is beyond the scope of this review.

#### THE PARATHYROID GLANDS

The technique of parathyroidectomy in pigeons has been described (86). The abnormalities in the human electroencephalogram in parathyroid insufficiency were corrected by parathyroid grafts (87). Parathyroidectomy in lactating rats (88) caused a decline but not cessation of lactation and no symptoms of tetany developed. Hollander & Riddle (89) studied a condition of melanism and parathyroid enlargement encountered in pigeons. McChesney & Giacomino (90) compared the efficacy of treatment of thyroid-parathyroidectomized dogs. With calcium salts tetany could be avoided but the serum calcium levels remained low. Parathyroid extract elevated the serum calcium. On a medium calcium and phosphorus diet, 2 mg. per kg. of vitamin D<sub>2</sub> or D<sub>3</sub> produced a rise in serum calcium to normal. Dihydratachysterol (1 mg. per kg.) produced as good or better effect. Tweedy (91) investigated

the effect of parathyroid extract on the distribution, retention, and excretion of radiostrontium. The extract caused retention of radiostrontium in the femurs and the kidneys, as well as a decreased fecal and increased urinary excretion of the labeled strontium. A decrease in serum calcium occurs in nephrectomized-parathyroidectomized dogs but not in nephrectomized controls (92), indicating that the parathyroid gland exerts an influence on calcium metabolism independent of the kidneys. The anatomical changes associated with the parathyroid hypertrophy which follows nephrectomy have been detailed by Baker (93).

#### THE GROWTH HORMONE

A full account of the isolation and chemical properties of pure anterior pituitary growth hormone by Li, Simpson & Evans (94) has appeared. This important work completes the identification of the major hormones as pure proteins and paves the way for greater accuracy in determining how the pituitary hormones are related to metabolism. The question of whether pure growth hormone is or is not the ketogenic agent of the anterior pituitary has not yet been ascertained. Becks *et al.* (95) have elicited the restoration of growth in the tibias of hypophysectomized rats with purified growth hormone. Bennett *et al.* (96) showed that growth hormone caused the same decrease in nitrogen excretion in rats with fractured femurs as in treated normal controls. The "protein catabolic period" after fracture occurred at a lower level of nitrogen excretion in the hormone-treated animals.

The effect of growth, aging, and diet on the lipid composition of rat tissue has been reported by Williams *et al.* (97, 98). All tissues increased in essential lipids during growth. Quantitatively, the fundamental change during growth, common to all tissues, was an increase in cephalin concentration. Horvath (99) has conducted a comprehensive study and review of the phosphate compounds of rat muscle during growth and aging. For example, adenosinetriphosphate was practically constant throughout life whereas maximal creatine concentration was not reached until the age of 120 days.

When crude diabetogenic pituitary extract was given daily to animals on diets just sufficient to maintain a steady body weight under normal conditions, nitrogen retention and increase in body weight were observed and, in the dog and cat, diabetes (100). The

nitrogen balance was positive during diabetes even when the D:N ratio was 3.6. Analysis of the tissues and of the carcass (rat) showed that much of the tissue added during pituitary treatment resembled muscle in its composition. The retained nitrogen was enough to account quantitatively for the added tissue. The metabolic rate was apparently not depressed. The loss of calories entailed by these processes was more than compensated by an increased oxidation of stored fat, and calculations of the weight and energy relations of fat and protein are presented to account for this, in agreement with earlier work (101). The essential concomitant action of insulin in achieving growth (N retention) is pointed out. In studies on the action of crude pituitary extract on growth in rabbits, the increased urine volume during extract treatment led Ogilvie (102) to infer that water retention was not a factor in the increased weight. This inference was not supported by Young's subsequent determination (100) of an increased water content of the tissues when growth hormone was given. Ogilvie concluded that the metabolic rate, although not necessarily the basal metabolic rate, was reduced. Whole pituitary extract increased the weight of islet tissue in the rabbit. Both Young and Ogilvie make no comment on the thyrotropic activity of the whole pituitary extracts used in their experiments, but the energy available from fat combustion presumably takes care of this aspect of the altered metabolism.

The capacity of growth hormone (Antuitrin G) to cause regeneration of the hind limb of hypophysectomized newts has been further studied by Richardson (103) who also noted the synergistic action of thyroxine on growth in this preparation. The retarding effect of estrone on the growth of rats was greater when the animals were on an iodine deficient diet (104). Zeckwer (105) found that although thyroidectomy retards the growth of the kidneys it does not prevent compensatory hypertrophy after unilateral nephrectomy. As there is a decrease in the number of acidophil cells in the pituitary after thyroidectomy, this means that kidney growth is not necessarily proportional to the number of acidophils. A recent compilation of the growth of domestic animals has been prepared by Brody (106).

*Steroid hormones and nitrogen retention.*—In addition to the growth hormone of the anterior pituitary, certain steroid hormones affect nitrogen metabolism. Those related to the androgens cause nitrogen retention and, having this feature in common with the

growth hormone, will be considered at this point. Others, related to corticosterone, accelerate nitrogen excretion and will be cited under the adrenal cortex. The need for a new appraisal of the hormones controlling protein metabolism is recognized but is not attempted here.

The cartilaginous growth produced by growth hormone in hypophysectomized rats was inhibited by large doses of testosterone (107). Herrick (108) found that female and capon fowls treated with testosterone for twenty days showed 100 per cent greater tensile strength of the skin and a 50 per cent increase in the breaking strength of the muscles due to increased fibrous tissue and collagen. Testosterone and stilbestrol given to castrated male and female rats did not change the serum calcium or phosphorus, nor the fecal excretion of these substances (111). The acid phosphatase in the serum was raised by castration and decreased, in intact male rats, by stilbestrol.

*Clinical studies.*—Reifenstein, Albright & Wells (109) have reviewed the methods used in their metabolic investigations on patients. Butler *et al.* (110) observing the effects of testosterone therapy on the metabolism of healthy subjects receiving an inadequate dietary intake, conclude that although this hormone conserves small quantities of protein under fasting conditions, it does not result in a significant saving of body substance as a whole. It apparently does not diminish the energy requirements, and by conserving protein it leads to a decreased gluconeogenesis and to an increased and less efficient utilization of fat. Nine androgenic steroids tested on immature boys brought about changes in nitrogen metabolism similar to those caused by testosterone but smaller and less well sustained (112). The studies in this field indicate that androgens and growth hormone affect nitrogen metabolism in quite different ways.

#### THE ADRENAL CORTEX AND ADRENOTROPIC HORMONE

*The adrenotropic hormone.*—The purification of anterior pituitary adrenotropic hormone by means of its solubility in warm alcohol has been studied (113). Both free amino and tyrosine groups are thought to be essential for its action (114). Pure adrenotropic hormone increased the resistance of rats to anoxia (115) and caused a fall in adrenal ascorbic acid (116) and a slower fall in adrenal cholesterol (116, 118). The level of these substances in other tissues

was not affected. In the adrenals, the time for the return to normal of ascorbic acid and cholesterol differed in the rat and guinea pig. An associated increase in liver glycogen was observed. Taken in conjunction with the earlier work the results provide a method for the bioassay of adrenotropic hormone. A preliminary report has been made on the assay of this hormone in blood under various conditions (117). Cortical extract prevented the decrease in adrenal ascorbic acid which follows exposure to cold but did not affect the decrease which follows exogenous adrenotropic hormone. The results indicate that, like other pituitary hormones, the release of the hormone tropic for the adrenal is regulated by the concentration of the adrenal hormones in the blood and tissues (119). There was no difference in the response to adrenotropic hormone of the intact and demedullated gland (120). The alkaline phosphatase in the plasma of rats is lowered by hypophysectomy (121), and it is also lowered in both normal and hypophysectomized animals by the administration of adrenotropic hormone. Ingle *et al.* (122) produced glycosuria in normal rats during the injection of pure adrenotropic hormone in doses which raised the adrenal weight to four times normal. With a comparable dose of adrenotropic hormone but without the forced feeding of carbohydrate used by Ingle *et al.* (122), an increased urinary nitrogen excretion in rats was observed, but only one of the animals had glycosuria (123). These results add further data on the known antagonism between the growth and adrenotropic hormones. An increase in adrenotropic hormone has been found in the urine of pregnant women (124).

*Chemistry of adrenal steroids.*—Since the synthesis of desoxycorticosterone intensive efforts have been made to synthesize steroid compounds with oxygen at  $C^{11}$  having the metabolic activity typified by corticosterone. Multiple methods of doing this have now been developed and their extension to commercial production is imminent. The problems of steroid chemistry are beyond the scope of this review but the partial synthesis of corticosterone must be mentioned (125). The work of the leading American investigators may be approached through a recent group of twenty-two articles (126 to 147).

Kuizenga *et al.* (148) have confirmed their previous conclusions that hog adrenal cortical extracts were rich in 11-hydroxylated steroids by obtaining the steroids in pure form. The most active steroid was present in six times the concentration in which it oc-

curs in beef adrenal extract. Thatcher & Hartman (149) prepared from adrenal glands a substance very potent in its ability to cause retention of sodium in the body. This substance differed from desoxycorticosterone in its chemical behavior. They recall the fact that cortical extract will replace the sodium-retaining function of the adrenal cortex but that none of the crystalline compounds which have been isolated in adequate quantities from the gland will account for its sodium retaining power. If their substance fills this gap its identification will be of obvious interest.

The methods of analysis of urinary 17-ketosteroids have been further described (150, 151), and three methods of determining 17-ketosteroids have been compared (152). Talbot *et al.* (153) have described a colorimetric assay for 11-oxycorticosteroids based on the method of Venning, *et al.* (154).

At present lack of the compounds rather than of methods prevents the study of the metabolism of adrenal cortical steroids. It is thus relevant to note related steroids which have been examined. Mason & Hoffman (155) have described the inactivation of progesterone by the liver and Miller & Dorfman (156) noted the conversion of administered  $\Delta^5$  androstenediol-3B-17a to dehydroisoandrosterone. From patients with adrenal hyperfunction new and closely related androstane derivatives have been identified in the urine (157, 158). A variety of other steroids were also identified (158). Extracts of human urine contain material which has an effect on potassium metabolism similar to that of adrenal cortical extracts and desoxycorticosterone acetate, as judged by a potassium intoxication test in adrenalectomized rats and a sodium excretion test in normal rats (159). Under the conditions of the latter test the adrenal cortical extract increases and desoxycorticosterone acetate decreases the excretion of sodium. One of the urinary fractions caused a small increase in sodium excretion, thus resembling adrenal cortical extract. Salter *et al.* (152) fractionated the ketosteroids in the urine of normal, endocrine and cancerous subjects and appraised the clinical significance of these methods. Lieberman & Dobriner (160) identified pregnanediol-32, 17-one-20 from the urine of various patients. The urinary 17-ketosteroid excretion of normal men was unaffected by prolonged wakefulness and slightly depressed by amytal (161). Reifstein *et al.* (162) made use of the fact that 17-methyl testosterone is not excreted as a ketosteroid to study its effect on the endogenous production of urinary

17-ketosteroids. When methyl testosterone was given, the excretion of ketosteroids decreased in several types of patients and this inhibition was attributed to a decreased production of some pituitary tropic hormone.

*The bioassay of adrenal cortical activity.*—The glycogen deposition and cold protection tests have been further studied during the past year. These tests, which measure the activity of the C<sup>11</sup> steroids, have been used to estimate the urinary excretion of this group of compounds. The sensitivity of the Reinecke-Kendall test, which is based upon the deposition of glycogen in the livers of glucose-treated adrenalectomized mice, has been increased by the administration of measured amounts of glucose to adrenalectomized mice (163). With this test, 11-dehydro-17-hydroxy-corticosterone was found to be three times as active as 11-dehydro-corticosterone, and it was possible to follow the daily urinary excretion of these substances in men. Dorfman *et al.* (164) described the conditions influencing the cold test and defined its statistical significance. The rat was more suitable than the mouse; the test was rapid and sensitive but was "marred by an erratic variation in sensitivity of animals from group to group." The number of animals needed for validity makes the test impractical for most purposes. The same critical methods have been applied (165) to the glycogen test as modified by Dobriner, and it was found that even when ten mice were used instead of five, the error might reach 150 per cent within the assay range of dosage. In this method glucose was not given and hormone prevented the fall in liver glycogen. Using both methods, Dorfman *et al.* (166) determined the relative potencies of several adrenal steroids. By the cold protection test they found that 17-hydroxy-11-dehydro-corticosterone was the most active and that 11-dehydro-corticosterone, corticosterone, and 11-desoxycorticosterone respectively were 1/3, 1/12 and 1/13 as active. By the glycogen test 11-dehydro-corticosterone was 1/4 as active as 17-hydroxy-11-dehydro-corticosterone, in good agreement with previous work (163). They comment on the difference in potency of a pig adrenal extract as measured by the glycogen test compared to that found by the survival-growth method. Four modifications of this glycogen test have been analyzed statistically (167), and in the most delicate but somewhat erratic method the glycogen levels were maintained by as little as 0.8  $\mu$ g. of Compound E. Palmer & Joseph (168) investigated the perfusion method of Hyman &



Chambers (169) for assaying adrenal cortical extract and found it unsuitable.

It seems reasonable to conclude that the survival of adrenalectomized animals remains the assay method of choice for adrenal cortical extracts. The glycogen deposition test, in spite of certain difficulties, has proved of value in the study of the urinary excretion of adrenal steroids in three laboratories as noted above.

*The adrenal cortex and enzymes.*—In an introductory paragraph Folley & Greenbaum (170) reviewed the brief literature on this subject. They confirmed the observation that adrenalectomy decreases liver arginase which was restored by desoxycorticosterone and to a less degree by  $C^{11}$  steroids. None of these compounds increased the arginase or alkaline phosphatase content of mammary gland which had been reduced by adrenalectomy. Only desoxycorticosterone elevated the kidney alkaline phosphatase levels of adrenalectomized rats. The renotropic effect of the androgens has been studied further by Kochakian (171) who also outlined the changes in acid and alkaline phosphatase in the kidney (172) in the course of renal hypertrophy. Kidney arginase has also been studied (173) and differences in the arginase response were observed when a 17-methyl group was present in the androgens. Undernutrition did not affect the ability of the steroids to stimulate arginase activity per gram of tissue. A further study of the effects of various hormones on renal structure (174) includes the effects of the adrenal cortical steroids.

*Other factors influencing the adrenal cortex.*—Ingle (175) has reexamined the effect of diet on adrenal weight in rats. When 80 per cent of the caloric value of the diet was derived from protein there was a definite increase in the weight of the adrenals, with 67 per cent protein there was little or none. He discusses the discrepancies which still remain between these results and those previously observed by others. Leatham (176) found that a 78 per cent casein diet did not cause adrenal hypertrophy, nor was the weight of the other endocrine glands altered although renal hypertrophy occurred. Vogt (177) observed the results of the chronic administration of epinephrine on the structure of the adrenal cortex. The changes consisted of an increase in the lipids of the cortex and, after 24 days, a significant increase in adrenal weight. The influence of ovariectomy on adrenal function was investigated by Smith (178) who found no important change in adrenal weight.



However the plasma sodium levels were higher; gluconeogenesis during fasting was increased, and histochemical studies of the cortex showed a decrease in the sudanophilic material. All of these changes occurred sixty to seventy days after ovariectomy and were then maintained. The evidence indicated an increased secretion of sodium-retaining and carbohydrate-active factors from the adrenal cortex in response to ovariectomy, and an increased secretion of adrenotropic hormone was postulated. On the other hand, Castillo & Rapela (179) found a decrease in adrenal weight one month after castration of female rats. The atrophy, like that following hypophysectomy, was more marked in the reticular zone. The injection of 10 mg. of desoxycorticosterone acetate daily for ten days was followed by a decrease in adrenal weight and atrophy of the whole cortex, which was also manifest when castrated animals were used. The adrenal-gonad relationship has been reviewed by Parkes (180).

*Other observations.*—Luft (181) has reviewed the subject of adrenal hyperfunction in man and Soffer's monograph (182) provides a well-written account of diseases of the adrenals. The subnormal plasma protein levels of adrenalectomized rats maintained on sodium chloride was shown by paired feeding experiments (183) to be due to the diminished food intake. Adrenalectomized rats maintained with desoxycorticosterone survived and grew on a carbohydrate free diet (184), but growth did not occur when fatty acids were removed and vitamin-free casein was used. In men subjected to sweating, 30 per cent less salt was lost in the sweat under treatment with desoxycorticosterone (185). Ralli & Graef (186) further studied the stimulating effect of adrenalectomy on melanin deposition in black rats and include data on the interaction of adrenalectomy, desoxycorticosterone, and vitamin B deficiency upon melanin metabolism. Schweitzer (187) has reported the effect of adrenalectomy on the contractile power of skeletal muscle in cats and dogs. The development of adynamia (fatigue) was associated with a fall in blood pressure and was reversible by blood transfusion. Muscular fatigue in these experiments was not relieved by prostigmine. According to Graham-Bryce *et al.* (188) the ingestion of pregnenolone did not improve the ability of subjects to perform prolonged psychomotor tests, in contrast to the studies of Pincus & Hoagland (189). The latter (190) have added a study of the effect of pregnenolone on factory workers. Performance was improved in some but not in others, and both groups of investi-

gators agree that benefit from the drug will be shown only by people who are under stress.

*Toxic effects.*—Selye *et al.* (191) studied the influence of electrolytes on the toxicity of desoxycorticosterone acetate. Sodium chloride and sodium sulfate increased the toxic effects whereas ammonium chloride, calcium chloride, and ammonium sulfate prevented these effects. Organ changes and biochemical findings were summarized. The nephrosclerosis produced by anterior pituitary extracts was prevented by the administration of ammonium chloride (192). Degenerative lesions were found in the cerebral arteries of a boy with Addison's disease who had received massive doses of desoxycorticosterone acetate (193). Harrison (194) attempted to reproduce the pathological changes in the joints, heart, and kidneys which have been attributed to desoxycorticosterone by Selye. He concluded that it was not possible to show that desoxycorticosterone was a factor in the causation of arthritis in rats and that infection, directly or indirectly, was concerned with the production of rheumatic cardiac lesions and nephrosclerosis. He recognized that a cold environment might also play a part and attributed the results of Selye to the effect of infection, alone or in combination with cold. However, desoxycorticosterone caused hypertension and aggravated the renal lesions of rats with experimental nephritis (194a).

*The adrenal cortex and lymphocyte response.*—The role of the adrenal cortex in antibody formation has been further studied (195). The rate of antibody production was increased in mice, rats, and rabbits when adrenal cortical extract was given at the time of antigen administration. In animals with established immunity, enhancement of antibody titer was induced by cortical extract due to the increased rate at which antibodies were released from the lymphocytes under the influence of the adrenal cortical hormones. Lymphocytopenia of stress fails to occur in adrenalectomized mice (196). After stress in normal men the lymphocyte count<sup>1</sup> falls and the ketosteroid excretion rises, whereas in psychotic patients the count rises and the rise in ketosteroids is erratic (198, 199). An analysis of the differential blood count in adrenal cortical diseases discloses a relative lymphocytopenia in Cushing's syndrome (hyperadrenocorticism) and a relative lymphocytosis in Addison's dis-

<sup>1</sup> See reference 197 for diurnal variation in lymphocytes.

ease (200). In summary, the lymphocyte response to stress in man, its correlation with ketosteroid excretion and the lymphocyte count in pathological disorders of the adrenal cortex all confirm the finding of earlier observers.

*Alarm reactions.*—The reaction to stress (alarm reaction; general adaptation syndrome) has been reviewed by Selye (201). The role played by the adrenal glands in the response to injury has long been recognized, and recent contributions concerning this have appeared. In a study of the factors concerned in the resistance to anoxia, Britton & Kline (202) suggest that the increased resistance of young animals may be due to the relatively large size of their adrenals. A high level of liver glycogen was associated with this resistance and larger adrenals were present in the more resistant females. The improvement of tolerance to anoxia by glucose administration and experiments on adrenalectomy, adrenal hypertrophy, and cortical extracts offered more evidence concerning the adrenal function in anoxia. In the case of nonfatal hemorrhage, Sayers *et al.* (203) observed that the fall in adrenal cholesterol and ascorbic acid resembled the changes occurring after the injection of adrenotropic hormone. The changes were prevented by hypophysectomy. Levin (204) likewise found that the adrenal cholesterol fell to half the normal level in rats subjected to anoxia, the serum cholesterol remaining unaltered. Cold and starvation caused smaller changes. Desoxycorticosterone caused irregular increases and stilbesterol a prolonged decrease in adrenal cholesterol. There was no protection from burn shock after treatment of rats with adrenal cortical extract (205). Splenectomy inhibited the pituitary-adrenal reaction to stress (206).

In recent years there have been numerous investigations on the metabolic response to injury or infection in man. The alterations are typified by the protein catabolic response manifested by a negative nitrogen balance, by a mobilization and negative balance of calcium, and by other changes. The subject is too large for review here, but the major sources of information are given as they have not been cited previously in these reviews. The role of the adrenals has been emphasized by Browne (207, 208) and Albright (209); the general metabolic response has been reviewed by Peters (210) and Cuthbertson (211) and the series of articles by Howard and his associates (212 to 216) present data on the mineral metabolism as well as on that of protein.

## INSULIN AND EXPERIMENTAL DIABETES

The monograph by Soskin & Levine (217) correlates the biochemical, physiological, and clinical aspects of carbohydrate metabolism. Other reviews have appeared on the etiology of diabetes mellitus (218, 219), the history of insulin (220), the pathology of experimental diabetes (221), alloxan diabetes (222), and phlorhizin glycosuria (223). The Houssays have reviewed the role of the thyroid (224) and of the pituitary (225) in diabetes.

The chemistry of insulin has been studied by a new method of identifying free amino groups (226) and the bearing of the results on the structure of insulin is discussed. The reversible inactivation of insulin by sodium lauryl sulfate (227) has been examined.

*The action of insulin.*—When Cori (228), in 1941, described the synthesis of glycogen *in vitro*, insulin had no effect on the reactions. This raised a discrepancy between the promotion of muscle glycogen by insulin in the intact animal and the failure of insulin to influence its formation *in vitro*. This discrepancy has been resolved by Price, Cori & Colowick (229). They found that the first step in the utilization of glucose, viz., the formation from glucose of glucose-6-phosphate by the action of hexokinase was inhibited by anterior pituitary extract. This inhibition was demonstrable either by the addition of pituitary extract *in vitro* or by treating rats with pituitary extract prior to the preparation of tissue extracts. Insulin counteracted this inhibition by pituitary extract although insulin alone had no effect on the hexokinase reaction. Rats made diabetic by alloxan yielded tissue extracts, the enzyme activity of which resembled that of rats treated with pituitary extract. Muscle extracts from diabetic rats after treatment with insulin showed normal hexokinase activity. These results indicate the close relation between this action of insulin *in vitro* and *in vivo*. The pituitary extract used was a fraction rich in growth and ketogenic activity. In addition, experiments with adrenal cortical extract have been reported (230). Cortical extract alone had no effect on hexokinase but greatly increased the inhibitory action of added or previously injected pituitary extract. Thus, when muscle extracts from diabetic rats were used, cortical extract produced a marked inhibition of hexokinase, which was attributed to an intensification of the action of some pituitary factor contained in the extracts of diabetic muscle. When inhibition by cortical extract was observed it was always released by insulin. The

adrenal factor was apparently one of the unknown compounds in the amorphous fraction, as Kendall's compounds A, B and E had no effect. In conclusion, these studies reveal at least one site of action of insulin in carbohydrate metabolism.

Although phosphate exchange is involved in the hexokinase reaction, Weissberger (231) found no change in the distribution of  $P^{32}$  in blood after the injection of insulin.

Present knowledge of the hexokinase reaction does not explain the insulin-sensitivity of the hypophysectomized animal, which has been re-examined by Bennett & Roberts (232). The insulin-sensitivity of hypophysectomized rats was increased still more by evisceration, and the protective effect of anterior lobe extracts against insulin hypoglycemia was demonstrable in the eviscerated hypophysectomized animals. They concluded that part of the hypersensitivity to insulin seen in hypophysectomized animals was an extra-hepatic phenomenon and their results also confirmed Himsworth's (233) work on the peripheral action of the anterior pituitary hormones. After control studies on the survival of eviscerated rats given saline solution (234), Ingle *et al.* (235) found that insulin greatly shortened the survival time. This effect was proportional to the dose of insulin and to the rate at which glucose was removed from the blood. De Duve (236) also investigated the action of insulin in normal, hepatectomized and eviscerated dogs, and emphasized the action of insulin on the liver. The action of insulin both in the liver and at the periphery is compatible since hexokinase and the phosphorylases have been found in both liver and muscle (228).

The fact that in the intact animal both pituitary extract and insulin increase glycogen formation does not necessarily conflict with the antagonism of these hormones observed in the hexokinase reaction. Both the glucose available to the tissues and its utilization by oxidation appear to influence the end result of the hexokinase reaction as reflected by glycogen levels. The findings (a) that pituitary extract does not cause an increase of liver glycogen in the depancreatized dog (237), and (b) confirmatory studies on the fall in muscle glycogen in hypophysectomized animals (238) point again to the need for both pituitary hormones and insulin in the maintenance of maximal glycogen levels. In hypophysectomized-adrenalectomized animals pituitary extracts main-

tained muscle glycogen but adrenal cortical extracts failed to do so (238).

Using the isotope technique, Stettin & Klein (239) have added strong support for the hypothesis that the glycogen present in the liver after the administration of adrenalin is formed from the blood lactate. After insulin, the increased muscle glycogen was apparently formed largely from glucose directly. They extend their earlier finding of impaired hepatic lipogenesis in diabetes, the deuterium content of the liver fatty acids serving as a direct measure of fatty acid synthesis. An increased rate of lipogenesis in response to insulin was not demonstrable in the rat which is relatively insulin-resistant. However, the liver fatty acids of rabbits treated with insulin contained four times as much deuterium as the normal controls (240). The rate of lipogenesis in the alloxan diabetic rabbit, like that in the rat, was distinctly subnormal. These articles provide new and important evidence that hepatic lipogenesis is retarded in hypo-insulinism and accelerated by the administration of insulin. The authors comment that the results do not necessarily imply a specific role of insulin in lipogenesis but that they may be secondary to an increased utilization of glucose in all its aspects.

Glucose was found to be very effective as a source of energy for intestinal smooth muscle contraction. Acetic acid was utilized equally well but fatty acids from propionic to pelargonic were less although still distinctly effective (241). The reviewer infers that the intestinal muscle, like the myocardium, can function in the absence of insulin because of its ability, shown in these experiments to use fuel derived from fat. Pretreatment with insulin decreased the resistance to anoxia (242), reduced the survival time of the isolated respiratory center (243), and decreased visual acuity (244).

Insulin resistance has been studied again by the impairment of the glucose tolerance test after intravenous typhoid vaccine (245). Felder (246) found an anti-insulin factor, which neutralized the action of insulin in mice, in the serum of a patient with severe insulin resistance. The inactivation of insulin by the administration of acetoacetic acid has been reported (247) but this preliminary work requires confirmation in view of negative studies of this type in the past.

The insulinotropic principle of the anterior pituitary is dis-

cussed by Conn & Louis (248) in connection with their observation that pituitary extract lowered the blood sugar of hypoglycemic patients. They give a valuable summary of the literature on this subject. Because the metabolic effects of pituitary extract have not been excluded as the stimulus to the islands, the reviewer is in tentative disagreement with their conclusion that there is an insulinotropic principle. Haist's review (249) indicates the complexity of this problem.

Simard (250) grafted the tail of the pancreas into the abdominal wall of two dogs. After two months one, a closed graft, had almost disappeared, but the other, prepared with a fistula, still contained many small islands. Hard *et al.* (251) correlated the glycogen and fat content of the pregnant, fetal, and new born guinea pig as indices of the activity of the fetal islets. Although there was an increase in fetal liver glycogen late in pregnancy, immediately after birth the liver glycogen and fat levels decline, and the R. Q. falls sharply to the fat level. These changes are the reverse of what is commonly expected in carbohydrate oxidation and lead one to infer that the pancreatic islands may have very little function at birth. When alloxan is injected into pregnant rats a positive test for alloxan is obtained in the fetal as well as the maternal blood (252). The mothers are diabetic but the litters are not, and the islands of the young show no pathological change. This suggests that only mature beta cells are susceptible to alloxan and that the islands are inactive until after birth.

Waugh *et al.* (253) report four cases of total pancreatectomy in man and give references to other recent reports on this operation in man affording the first opportunity for comparison with other species. This could best be done by the fasting glucose and nitrogen and acetone body excretion, but such data are at present too meager to discuss. Apparently the depancreatized man resembles most nearly the depancreatized dog. In this article Sprague discusses the insulin requirement after pancreatectomy, and points out that the somewhat lower insulin requirement of the depancreatized human being does not necessarily signify that the physiologic mechanisms are fundamentally different from those of ordinary diabetes.

Diabetic rats select less carbohydrate and more fat, the former falling from 65 to 30 per cent of the total calories ingested, and the latter increasing from 15.2 to 38.8 per cent (254). On this selection



they gained weight, were free of diabetic symptoms, and showed normal activity. When they were returned to a mixed diet and hence forced to eat the various ingredients in fixed proportions, diabetic symptoms and decreased activity recurred. In addition to developing cataracts on the stock diet, most of the diabetic rats had enlarged cecums. The impairment in fat formation in diabetic rats is another possible stimulus to such a dietary adaptation (240).

*Pituitary-diabetes.*—The effects of diabetogenic anterior pituitary extracts on growth have been noted (100). Such extracts caused transient diabetes and islet hypertrophy in rabbits (255). Gray & Oakley (256) failed to demonstrate any contra-insular action in the plasma of diabetic patients, in agreement with Dohan (257). Intercapillary glomerulosclerosis was found at autopsy in a dog with pituitary diabetes of five years' duration (258). The production of glycosuria in normal rats by means of pure adrenotropic hormone has been noted (122). Ingle *et al.* (259) induced glycosuria in rats by forced feeding and the administration of 5 mg. daily of C<sup>11</sup> steroids, and compared the results with the diabetes of partially depancreatized rats. In both types of diabetes the onset of glycosuria was accompanied by loss of weight and increased nitrogen excretion. These abnormalities were reversed by stopping the treatment with steroids or by giving insulin to the depancreatized animals. However, during adrenal steroid diabetes the animals were highly resistant to insulin. Insulin did not lower the nitrogen excretion during adrenal steroid diabetes even when the glycosuria was largely controlled.

The influence of high and low carbohydrate diets on the respiratory exchange after glucose has been studied, and the impairment of glucose utilization associated with a low carbohydrate regime was indicated (260). Glycosuria does not increase the urinary excretion of chloride in patients without ketosis (261). "Amelin," claimed to be antidiabetic (262), has since been reported to be ineffective in man and cats (263).

*Alloxan diabetes.*—Since the discovery of experimental alloxan diabetes a number of methods for the determination of alloxan have been developed. Leech & Bailey (264) first reported that alloxan added to blood *in vitro* and after intravenous injection in rabbits disappears completely in five minutes or less. They used a ferricyanide method with the accuracy of blood sugar determination. Archibald (265) described six methods for measuring alloxan



in quantities ranging from 0.02  $\mu$ g. to 2 mg. This article gives a comprehensive account of the chemical problems. The blood of normal men and dogs contained less than 0.02 mg. of alloxan per 100 cc., and the rapid disappearance of alloxan added to blood was confirmed. Absence of alloxan in normal and diabetic blood and its rapid disappearance after injection has been confirmed using a fluorometric method (266). Two additional methods have been reported (267) suitable for 0.05 to 4  $\mu$ g. quantities. In contrast to the above failures to identify alloxan in blood, two qualitative tests have yielded positive results in most of the tissues studied (268). The changes undergone by alloxan in the body are not yet understood and the disappearance of alloxan as such is so rapid that its possible role in human diabetes remains unsettled. The chemical structural requirements for diabetogenic activity have been summarized (222, 269, 270). At the present time six compounds, viz., alloxan, methyl alloxan, alloxantin, dimethyl alloxantin, dialuric acid and methyldialuric acid, have been found to be diabetogenic by one or more investigators, but twenty-three other related compounds have not, indicating the remarkable specificity of alloxan as a toxin to the beta cells.

The diabetogenic potency of alloxan is rapidly lost after injection (271) as shown by protection of portions of the pancreas in which the circulation was occluded for periods of from one to six minutes after the injection of alloxan. In addition to pancreatic lesions in rabbits, degenerative cytological changes in the basophilic cells of the anterior pituitary and in the cells of the adrenal cortex have been described (272). This is interpreted as part of the general cytotoxic action of alloxan and not as evidence that the diabetogenic action is exerted indirectly.

The general problems of alloxan diabetes have been the subject of a number of papers: the dwarfing effect of alloxan diabetes in rats (273), the changes in plasma and liver phosphates in diabetic coma produced by alloxan in rats (274), and the relation between the dosage of alloxan and the severity of diabetes to the development of hyperlipemia in rabbits (275).

For the more consistent production of diabetes, Duffy (276) gave glucose and insulin after 200 mg. per kg. intravenously in rabbits. Kass & Wasibren (277) produced diabetes in 90 per cent of rats previously starved for forty-eight to sixty hours and then injected with 175 mg. per kg. of alloxan. Only 25 per cent of rats

which had not been starved became diabetic after this dose of alloxan. Diabetes was produced by feeding alloxan to rabbits and to cats (278, 279).

Prolonged subdiabetogenic doses of alloxan impair carbohydrate metabolism (280) as shown by the glucose tolerance test. Doses of 25 mg. per kg. led to progressive decline in tolerance. The initial hyperglycemia was not observed when alloxan was given to adrenalectomized or hypophysectomized rats (281). The inactivation of the adrenals abolishes the initial hyperglycemic phase of alloxan in cats (282), but adrenalectomy failed to do this in dogs in which removal of the liver was required (283). Pancreatic grafts fail to secrete insulin after alloxan (283). The influence of partial pancreatectomy upon the response to alloxan has been investigated (285), this operation having no effect on the hypoglycemic phase of alloxan injection in rats. On the other hand, Banerjee (286) found a diminution of the hypoglycemic phase in partially depancreatized rabbits given alloxan.

More articles have appeared on the action of alloxan in different species. The pancreas of the toad (287, 288) is not injured nor does diabetes develop. Hyperglycemia was produced in the turtle (289). In the frog (290) changes in the beta cells occurred more slowly than in mammals, and complete necrosis of the islands did not occur. The injection of 200 mg. per kg. of alloxan in the duck and chicken (291) produced no change in the blood sugar level, although extensive necrosis of the islands was reported. After alloxan was administered intravenously to barn owls, horned owls, pigeons, ducks, and chickens, there were no significant blood sugar changes except for slight and irregular hyperglycemia in pigeons (292) nor was pancreatic pathology observed except for slight changes in occasional beta cells comparable to those previously reported (293). The finding of crystalline deposits on the serosal surfaces in pigeons confirmed the results of Goldner & Gomori (293) who had identified this as sodium urate.

The oxidation of glucose by the brain of alloxanized rats *in vitro* has been studied, but adequate criteria indicating the absence of insulin in such animals were lacking (294). In general, the results conform to those obtained after pancreatectomy. The tendency of animals with alloxan to develop thiamine deficiency was the same or slightly less than the controls (295). These results oppose the hypothesis that the requirement of thiamine is in-

creased in diabetes. They appear applicable to diabetes mellitus because in alloxan and human diabetes the insulin deficiency is commonly partial and, in both, fat absorption is unimpaired.

The role of the endocrine glands in alloxan diabetes has been further investigated. Bailey *et al.* (296) produced diabetes and the usual necrosis of the islets by giving alloxan to hypophysectomized rats, thus showing that the effects of alloxan were not mediated by the pituitary. Houssay & Sara (297) state that hyperthyroidism increases and thyroidectomy decreases the sensitivity to the diabetogenic action of alloxan, but in both cases hyperglycemia was produced by the doses commonly used.

Amelioration of diabetes after total adrenalectomy is apparently due to the reduced food intake rather than to adrenalectomy per se (298, 299), confirming older studies on partial pancreatectomy or pituitary diabetes in which some insulin is still present. Stilbestrol is neither diabetogenic nor beneficial to rats with alloxan diabetes (300).

Finally the protection of animals from alloxan diabetes may be summarized. The protective influence of feeding has been noted (277). Inhibition of the action of alloxan was observed in animals treated with 3,4-diaminotoluene, orthophenylenediamine and sodium bisulfite (301). By the intravenous administration of glutathione or cysteine one to two minutes prior to giving alloxan, Lazarow (302) completely protected rats from diabetes. He notes that his dosage of glutathione was much larger than that used by Leech & Bailey (264) and his doses of cysteine were larger than those used by Weinglass *et al.* (301). Other amino acids, ascorbic acid and phosphate buffer afforded no protection. When the sulfhydryl compounds were given three or more minutes after alloxan there was no protection. For further details, Lazarow's well-documented discussion is recommended.

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THE GEORGE S. COX MEDICAL RESEARCH INSTITUTE  
UNIVERSITY OF PENNSYLVANIA  
PHILADELPHIA, PENNSYLVANIA

# THE PHYSIOLOGY OF SUPPORTING TISSUE<sup>1</sup>

BY P. D. F. MURRAY

*Department of Biology, St. Bartholomew's Hospital Medical College,  
London, England*

Earlier reviews are those of Huggins (1), Newton (2), McLean (3), Dallemagne (4), and Leriche (5), which deal with the bone cell and with bone in wider problems of physiology and pathology. On more restricted subjects may be mentioned Logan (6) and Sendroy (7) on mineral calcification and mineral metabolism.

## THE FINE STRUCTURE OF BONE

X-ray diffraction studies have been reviewed (8). Studies of the birefringence of bone (9, 10) led to the conclusion that its microscopic architecture is related to the vascular pattern rather than to external form or mechanical function.

## THE GROWTH AND MECHANICAL STRUCTURE OF BONES

The architecture of the human femur could be produced as a pure growth structure, function playing no necessary part (11), but alterations in the architecture are in accord with mechanical principles (12). Muscular forces play a part in the development of torsion in the humerus (13, 14).

Endochondral growth in length of long bones in the rat (15), and growth of the epiphyses (16) were studied. Growth in length of long bones (17) was said to be predominantly periosteal (18) and predominantly endochondral (19 to 21); the causal relationship between the events in endochondral ossification were analyzed (19 to 25). The role of functional and other factors in the development and maintenance of structure were studied in the jaws and teeth of human foetuses, and in form changes of the edentulous mandible (26). The development and function of the organic component of enamel (27), and of cementum (28), and the relations between ameloblasts and odontoblasts were investigated.

## THE EVOCATION OF BONE FORMATION

Alkaline phosphatase, mixed with calcium glycerophosphate in a sodium alginate gel, induced ectopic ossification in the muscles of

<sup>1</sup> This review covers the period from 1943 to 1945, approximately.

rabbits (30). Bone, developed from the local connective tissue, formed around the transplanted central parts of growth cartilage of very young rabbits (20, 21, 23, 25). An alcoholic extract of the cartilaginous epiphyses of the long bones of newly-born rabbits induced in the thigh muscles of others the development of large bony and cartilaginous masses—which, apparently, had the general structure of a long bone (25). The observation approximates to a confirmation of the induction of bone formation by alcoholic extracts of bone (31, 32).

#### THE REPAIR OF FRACTURES

Dallemagne (33 to 35) finds in bone repair in rabbits that: (a) there is a demineralization of both stumps of the broken bone, preceded in the proximal piece by an accumulation of mineral in the first few days after fracture; (b) there is a similar but lesser demineralization in the intact homologue of the fractured bone (confirming Roche); and (c) in other bones there are only occasional and irregular variations in mineral content. The skeletal demineralization stops long before the repair tissue is fully calcified, which must therefore obtain much of its mineral from other sources. The demineralization is attributed to vasodilatation and resultant local acidity which not only has its own decalcifying action, but intensifies that of the parathyroid hormone.

Fracture repair is described in rats; thyroxin and stilboestrol, in spite of their activity at the growth cartilage, have but little effect on events at the callus (36). Rachitogenic diets (37, 38), and vitamin A deficiency (38), greatly delay or prevent the repair of fractures in rats. In pigeons, mineralization of the callus is accelerated by parathyroid hormone and vitamin D (39, 40).

All authors agree that deficiency in vitamin C retards or inhibits the repair of bone. The developing connective tissue callus is described in normal guinea pigs as enriching itself in ascorbic acid, which is again reduced in quantity when calcification starts (41, 42). Injections of calcium ascorbate produced more bone than ascorbic acid or calcium gluconate (43, 44). Both endosteum and periosteum took part in bone repair (45).

Earlier observations that there is a loss of endogenous nitrogen from patients recovering from fractures are confirmed (46, 47). The repair of experimental bone defects in rabbits is accelerated by filling the gap in the bone with alkaline phosphatase and calcium glycerophosphate in sodium alginate (30). Similar results have been

obtained with a paste of salts in the same proportion as in bone, in a gelatin base (48 to 50).

#### THE NATURE OF THE MINERAL SUBSTANCE IN BONES AND TEETH

The widely-accepted opinion that the mineral component of bone is an apatite of one sort or another has been rejected in favor of the view that it consists of tricalcium phosphate and calcium carbonate not in chemical combination with one another (4, 35, 51, and references there given). The extensive evidence includes comparative studies of the effects of heat on bone salts on artificial salt mixtures, and on apatites, as well as differential solubility studies, examination of x-ray diffraction spectra, of specific gravities, and of refractive indices. The mineral of dentine and dental cement was held to resemble that of bone but in enamel the prisms consist of carbonato-apatite, while the interprismatic substance is impregnated with tricalcium phosphate (4, 51 to 53). It was, on the other hand, argued (54), from the constancy of the ratio calcium to phosphorus to carbon dioxide throughout the calcification of enamel, that a single complex compound is deposited, but that this is not an apatite.

The relation between the bone salts and the organic matrix was attacked by a study of the birefringence of bone (4, 9, 10, 35) and by other means; the authors concluded that in natural bone the tricalcium phosphate is not hydrated, but that the place of the water of crystallization is taken by some organic radicle to which is attributed a short chain of three or four carbon atoms.

The view that the salt first formed in calcification might be bicalcium phosphate has received support. Thus, in the intramedullary bone formed in birds under the action of oestrogens, the ratio of calcium to phosphorus is between 1.29 (the figure for bicalcium phosphate) and 1.94 (that for tricalcium phosphate), suggesting that the bone contains a mixture of the two salts. The authors found evidence that the less salt had been deposited, the closer the ratio was to that for bicalcium phosphate; they therefore consider that the first mineral deposited consists of this salt, but that it is speedily transformed into tricalcium phosphate (55). In the developing bones and teeth of mammals, amphibians and selachians (56 to 58), in fracture callus before it hardens (59), and in repair bone formed under a raised periost (60), and in the bone formed by rats on rachitic diets low in calcium (61), similar evidence was found. In the diaphyseal bone of chick embryos, the



ratio of calcium to phosphorus is 1.8, indicating tricalcium phosphate with apparently no admixture of calcium carbonate (62).

#### CALCIFICATION

The chemical problem of calcification has been dealt with in earlier reviews (2, 3, 6). It was emphasised (61, 63 to 65) that, whatever part living cells may play in calcification, the composition of the mineral substance depends on that of the liquid phase. When calcium, magnesium, phosphate, bicarbonate, and citrate ions are present in artificial solution in the amounts found in serum ultrafiltrate (63, 64), they are contained in the precipitate in approximately the same ratio as in bone or dentine, but when their amounts in the liquid resemble those in saliva, their ratio in the precipitate is like that in dental enamel. Studies of the mode of nutrition and of the lymph flow in teeth showed that both enamel and dentine may be supplied from the saliva as well as from the blood (66 to 68). When solubility measurements were made using large amounts of solid, the quantities of calcium and phosphate dissolved were much less than were present in the diffusible form in blood, but both magnesium and citrate increase the solubility. Precipitates formed from solutions containing citrate, magnesium, bicarbonate, and chloride, in the amounts found in blood, were for a few days roughly as soluble as the inorganic calcium and phosphate of serum (64). The uptake of sodium and phosphate by bone, dentine, and enamel has been studied *in vitro* (69 to 71). Diffusion was probably a controlling factor.

An investigation of the bones of young rats on rachitic diets (65) showed that bone carbonate depended on the diet and more immediately on the composition of the serum, especially on the ratio of serum carbon dioxide to serum inorganic phosphorus. Weinmann has published an important series of papers on the calcification of teeth (72 to 76) (see also 77). The distribution of alkaline phosphatase has been examined in the tissues of guinea pigs (78), in the chick embryo (79), in developing teeth (80), while Gomori (81) has studied histochemically its relation to calcification in the embryo, in a rachitic rat, and in the developing ectopic ossification induced by transplanted urinary bladder mucosa.

In the cyclically growing scales of fishes, phosphatase activity is high during active bone formation (82, 83), but is greatly reduced when the scale is growing slowly. The enzyme is present just before

and during calcification of the bones and teeth of embryonal mammals (57, 58) and in fracture callus (41, 42). It has recently been suggested that phosphatase may perhaps play a part in the development of collagen fibers (84), and hence of the organic component of bone as well as in its calcification. Thus, it is present in the newly-formed connective tissue of healing skin wounds, but is reduced or absent from that of the nonhealing wounds of vitamin C deficient animals. Several authors (78, 85 to 88) have found in vitamin C deficient guinea pigs a considerable reduction in serum- and bone-phosphatase, but a much smaller fall, if any, in the soft tissues; it was generally agreed that ascorbic acid is essential for the development of the connective tissue matrices.

Roche and his school have proposed a theory of calcification somewhat different from those of Freudenberg & Gyorgy and of Robison. According to Roche, calcification proceeds in four stages: (a) phosphate and calcium ions are fixed simultaneously but independently to the "pre-osseous substance"; (b) the pre-osseous substance undergoes a transformation whereby it becomes the organic matrix of the bone, and the calcium and phosphate ions are liberated; (c) these combine, an insoluble calcium phosphate is precipitated; and (d) is fixed to the proteins of the organic matrix. The first stage involves the activity of phosphatase which provides the phosphoric ions. The evidence supporting this theory was put forward in a series of papers dealing with the uptake of ions and the activity of phosphatase in fracture callus (40), in embryonal bones (56, 57), in ossifiable and nonossifiable parts of embryonal long bones calcifying in artificial solutions (58), and was summarized by Roche & Deltour (89, 90). The removal of phosphate from a medium containing bone and glycogen appeared not to be enzymatic because it occurred after heating the bone to 100° C. for half an hour (91).

#### ENDOCRINE GLANDS

*Endocrines and skeletal growth.*—The histological changes at the growth cartilages of hypophysectomized rats have been studied (92, 93). The Silberbergs' studies of the effects of the growth and other hormones on senescence are considered separately below. The body weight and skeleton of thyroidectomized new-born rats grow extremely slowly, and the appearance of ossification centers and eruption of teeth are delayed (94); injections of such animals with

growth hormone accelerates growth but not the formation of new ossification centers (95). The changes in proportion seen in the skulls of growing animals after thyroidectomy have been studied (96). Testosterone propionate injected into hypophysectomized female rats gave some reactivation of the growth cartilage. Injections of growth hormone increased both body weight and skeletal dimensions, and these effects were increased by simultaneous injections of testosterone. The two hormones thus act to some extent synergically (97).

Injection of the adrenocorticotrophic hormone narrows the growth cartilage and retards osteogenesis, but both effects are less extreme than after hypophysectomy (98). Adrenocorticotrophic hormone prevents the restoration of growth to hypophysectomized rats by growth hormone (99, 100). It was suggested that the antagonism may reflect the opposite metabolic effects of the two hormones. That the adrenocorticotrophic hormone acts through an adrenal hormone was shown by its inactivity in adrenalectomized animals (101). Adrenalectomy does not interfere with the restoration of growth in hypophysectomized rats by means of growth hormone (102). Disuse atrophy of the skeleton is increased by castration and reduced by either oestradiol or testosterone propionate (103).

*Endocrines and mineral metabolism.*—The controversy on the mode of action of the parathyroids continues. The most important factor determining the size of the glands seems to be the dietary or serum calcium (104 to 107). Several authors have studied the effect of nephrectomy on parathyroid action. Contrary to earlier findings (108), it was stated that parathormone raises serum calcium in nephrectomized animals in the usual manner (109) and it was found (110) that injections of the extract induced in nephrectomized rats an osteitis fibrosa which was distinguishable from that caused by the nephrectomy. Finally, it was found (111) that the parathyroids maintain a normal serum calcium after nephrectomy. Thus, it seems likely that the parathyroids may act directly on the bones; this need not necessarily exclude an action through the kidneys.

*Endosteal bone formation.*—The influence of oestrogens and androgens on the skeleton has been reviewed (112). The histology of the oestrogen-induced endosteal bone has been studied in birds (113 to 115), rats (116), and mice (117). The relation between the normal endosteal ossification and the reproductive cycle has been

studied in pigeons (119) and other birds (120). The medullary bone disappears more or less completely after cessation of injections (118), and in the nonreproductive period. Neither a rachitogenic diet in mice (121), nor parathyroidectomy in birds (122) prevented the formation of endosteal bone after oestrogen treatment.

The oestrogen injections cause hypercalcaemia and the new bone is more heavily mineralized than the old (123). The additional mineral is exogenous, and not of endogenous origin as is apparently the case in rats (116), for the oestrogen raises the intestinal absorption and the retention of calcium (124). On a calcium-deficient diet (125), the endosteal bone is imperfectly calcified, its mineral component being supplied by osteoclastic resorption in the old bone, and the parathyroids enlarge. The parathyroids were said to be essential (126, 127), and unnecessary (122, 128, 129), for the appearance or persistence of the hypercalcaemia caused by an oestrogen. Loss of the thyroid was said (130) not to have affected the hypercalcaemia and ossification induced by oestrogens. Thyroxin prevented the hypercalcaemia and other blood changes but not the endosteal bone formation (131). Removal of the anterior pituitary reduced the rise in blood-calcium and also the fall attributed to parathyroidectomy (132). The blood changes in oestrogen-treated birds have been intensively investigated (122, 128, 129, 131, 133). The increases in blood-calcium and blood-phosphorus are in the nonultrafiltrable fractions, chiefly as serum-vitellin to which most of the calcium is bound, and as colloidal tricalcium phosphate. The effect of parathyroidectomy is on the ultrafiltrable phosphate and calcium (128).

*Age changes.*—M. and R. Silberberg have continued their studies of the influence of endocrine glands on skeletal senescence. Growth hormone (134), parathormone (135), potassium iodide (136), and breeding (137), all promote changes characteristic of skeletal ageing (calcification and break-down of the growth cartilage, formation and resorption of bone, epiphyseal-diaphyseal union, etc.). Potassium appears to act directly, not through the thyroid. The actions of thyroxin and stilboestrol were compared (138); the effect of thyroxin is more accelerative, that of stilboestrol more depressive. When an oestrogen and anterior lobe extract are simultaneously administered, each hormone tends to exert its own effects (139).

*Endocrines and the embryonal skeleton.*—The inter relations of endocrines in embryonal life, and their relation in chick embryos

to achondroplasia, were discussed (140, 141). Evidence was found in tissue culture experiments (142, 143) that the plasma and tissue juices of older embryos promoted the differentiation of skeletal tissues in explants failing to differentiate in media from embryos of their own age or younger. Tissue of the anterior pituitary gland, cultivated with osteogenic cells, promotes ossification.

#### NUTRITION (EXCEPT VITAMINS)

Work in the field of general nutrition (144 to 147) showed how the form of bones (and indeed of whole animals) depends not only on the level of general nutrition, but on the time in the growth period when the nutrition was good or bad.

*Manganese and perosis.*—The changes caused by manganese deficient diets in rabbits (148), rats (149 to 151), and mice (150), may include decreased ash, lowered breaking strength of bones, limb deformities and other changes interpreted as the results of suppression of osteogenesis (148). Excess dietary manganese restricts growth and, by increasing faecal phosphorus and calcium, may lead to rickets (152). Injection of manganese chloride causes enamel hypoplasia in rats (153). In poultry, two vitamins of the B group, choline and biotin, and probably one or more in yeast, as well as manganese, are necessary for the prevention of perosis (154).

*Fluorine.*—The bone changes caused by fluorine have been studied in farm animals (155), and in rabbits (156). An association has been noted between dental fluorosis in children and such skeletal abnormalities as spondylosis deformans (157). The action of fluorine in preventing or postponing dental caries has been repeatedly confirmed. It was suggested that the action may be enzymic (158), and (159) that the carbonato-apatite of the tooth may be converted into a less soluble fluor-apatite. Evidence was presented to show that the effects of fluorine on teeth are mitigated by calcium (160). The calcification changes caused in the teeth by fluorine have been studied histologically (161).

*Magnesium.*—The slow return of magnesium to the depleted teeth has been studied (162).

*Amino acids.*—Deficiency of lysine in the diet of rats causes hypoproteinaemia and stops growth; there is reduction of the growth cartilage and imperfect mineralization of the bone (163).

*Effects of pregnancy and lactation on the skeleton and teeth.*—In rats, mineral deposition is normal during pregnancy and the

bones grow both in the spongy bone and in the shaft, but during lactation, shaft growth may be suspended, and, at the bone ends, there may be actual loss of weight and decrease of ash (164). The young of calcium-deprived rats had bones very low in ash, but they gained a little during lactation (165). In man (166), the chief factor determining the degree of calcification in the bones of infants during the first six months of life is the amount of calcium stored in the foetal skeleton. Skeletal abnormalities are reported in the offspring of rats on a rachitic diet; they are prevented by vitamin D (167).

#### VITAMINS

*Vitamin A.*—In vitamin A deficiency of puppies, the distribution of osteoblasts and osteoclasts in the skull and vertebral column is altered; the two kinds of cell may even change places. The bones are abnormally thickened and, by pressing on the brain, spinal cord, or nerves, cause the characteristic inco-ordination (168). Another report (169) attributed to the nervous system the blame for the disproportionate growth of the nervous system and the bone.

In hypervitaminosis A of rats (170), characteristic lesions are haemorrhages and, in young animals, fractures; there was no significant change in the ash content of the bones. The resemblance to scurvy was shown to be superficial, and the hypothesis that vitamin D protects against large doses of vitamin A was tested and not confirmed.

*B vitamins.*—A series of gross abnormalities involving the skeletons of rats were found to be caused by deficiency in riboflavin (171). (See also under "Manganese and perosis.")

*Vitamin C.*—Abundant formation of collagen fibers was reported in various tissues cultivated in the plasma of scorbutic animals plus embryo extract, while the activity and multiplication of cells was reduced (172 to 174). So unexpected a result requires confirmation. It was claimed that the lessened ability of the cartilage of scorbutic animals to fix toluidin blue or thionin is due to their failure to form chondroitin sulphuric acid, and that the weakness of the connective tissue may be due to its inability to form this or mucoitin sulphuric acid (175). In the bones of guinea pigs vitamin C deficiency affects neither the percentage of organic matter, ash, calcium, phosphorus, nor the calcium to phosphorus ratio (176).

The classical picture of scorbutic lesions is connected with the mechanical strains of movement (177), for immobilized extremities are without the usual micro-fractures and debris, but have a wide lattice of cartilage trabeculae on which no bone is deposited, and the marrow remains haematopoietic. The structure and development of a subperiosteal hyperostosis, previously reported by Mouriquand, is described, and is attributed to mechanical detachment of the periosteum from the bone surface (178, 179). Changes closely resembling those of scurvy, but neither prevented nor cured by ascorbic acid, can be produced by gluco-ascorbic acid in the diet of mice and cotton-rats (180).

#### VITAMIN D AND MINERAL METABOLISM

The size, strength and ash content of the femora of rachitic rats are smaller than in nonrachitic controls, but the calcium in the bone ash and the ratio, bone calcium to phosphorus, remains unchanged (181). A long-continued low intake of calcium causes osteoporosis accompanied by crushing and destruction of parts of the skeleton (182). The retardation of the growth of rachitic rats occurs in the normally noncalcified parts of the growth cartilages and probably in the soft tissues; it is quite distinct from the effects of the deficiency on calcification (183). In rachitic rats there is a failure of alveolar bone to resorb with resulting injury to periodontal tissue by compression (73).

Evidence accumulates against the view that vitamin D acts solely by influencing absorption. It was found (184, 185) that the effects of vitamin D on calcification and on calcium retention are distinct. Rats injected with radio-calcium or radio-strontium retain more of these substances if supplied with vitamin D than if the vitamin is withheld; this could not happen if the vitamin acted only in the intestine (186). The intestinal loop method, used in a manner which was claimed to eliminate errors held to vitiate the work of Nicolaysen, gave opposite results from his, vitamin D being without influence on the absorption of calcium (187). (See also 188, 189.) Alkalosis was said to be the immediate cause of rickets (190).

#### CARTILAGE AND CONNECTIVE TISSUE

Some aspects of the physiology of cartilage have already been discussed; for example, the action of growth hormone is partly on the growth cartilages. The structure of hyaline cartilage has been



examined and related to its mechanical functions (191, 192). The histogenesis of cartilage has been studied in a transparent chamber in a rabbit's ear; it is formed in regions of slow or moderate circulation (193). A useful chemical review (194) was given of studies on mucoitin and chondroitin sulphuric acids, on hyaluronic acid, and on heparin; the chemical and physical properties of these substances have been studied (195, 196). The nutrition of Wharton's Jelly, cartilage, and cornea have been discussed (197).

As human costal cartilage ages from infancy to the fourth decade (198), the chondroitin sulphuric acid at first increases but later decreases as calcification increases. The fibrils appearing are probably not new formations but are revealed by the disappearance of the polysaccharide between them, and this is also partly responsible for the lowered resiliency of the matrix. Collagen fibers have been studied with the electron microscope (199).

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DEPARTMENT OF BIOLOGY  
ST. BARTHOLOMEW'S HOSPITAL MEDICAL COLLEGE  
LONDON, ENGLAND

## MUSCLE<sup>1</sup>

BY FRITZ BUCHTHAL

*Institute of Neurophysiology, University of Copenhagen, Copenhagen, Denmark*

In reviewing an extensive field such as muscle physiology it is necessary to limit the subject. Consequently, work on energy metabolism, muscular exercise in the intact animal, and muscle pathology will not be considered. The review will deal mainly with skeletal muscle, as the physiology of smooth muscle has recently been treated in detail by Fischer (1) and Postma (2), and investigations on heart muscle will only be discussed in so far as they allow a comparison with skeletal muscle. Unfortunately, another limitation is imposed on this review by the fact that a number of important American publications are not yet available in formerly occupied European countries. An attempt has been made to compensate for this deficiency by extending this review to a number of European papers of recent date which had to be omitted from the preceding Reviews.

*General considerations.*—Research on muscle physiology was first mainly concentrated on the study of chemical processes as observed in more or less complicated enzyme systems (muscle extract, muscle debris) and their relation to the thermodynamics of the intact muscle. These studies led to a relatively complete picture of the chemical reactions involved in the energy supply of muscular activity and made it appear probable that all chemical processes known hitherto were restitutional processes. A decisive stage in the historical development was the extension of studies to the structure of the substrate, i.e., the structural elements of muscle. Work was concentrated with renewed interest on both the protein chains of myosin, which were assumed to represent the contractile element, and the minute structural properties of muscle fibres in relation to physiological reactions. Especially from the latter experiments it is quite evident that work on isolated fibres has in many instances facilitated the interpretation of experimental results.

The interpretation of chemical reactions as restitutional processes and of oriented protein chains as the structural basis of

<sup>1</sup> This review covers the literature to April 1946.

contraction leads to a concept regarding the distribution of energy-supplying processes during rest and contraction which is quite different from that of the "lactic acid period." Muscle structure at rest is considered to be the site of potential energy which is discharged during contraction. A promising initial stage in the establishment of the "missing link" between the chemical processes, as studied in muscle extracts and the minute structural elements of the muscle fibre, was the discovery of a close interaction between myosin and adenosinetriphosphate (ATP). This has been substantiated in work on myosin threads, as well as in work on the isolated muscle fibre.

#### MECHANICAL PROPERTIES

*Length-tension (stress-strain) diagrams.*—Both in whole muscle (3) and in the isolated fibre (6) resting tension over a considerable range of length is an exponential function of stretch. Reliable information with respect to static mechanical properties of the contractile substance can, however, only be obtained by investigation of the isolated uninjured muscle fibre. While there is agreement with respect to the shape of these length-tension diagrams at rest and during contraction, the rôle of the sarcolemma in the mechanical tension of the resting fibre is still an object of discussion. As empty sarcolemma tubes are claimed to have a length-tension ratio similar to that of intact parts of the fibre, tension in the resting fibre has been ascribed to the sarcolemma (4), while other investigators (5, 6) localise it to the substance inside the fibre. In the reviewer's opinion this question requires further experimental investigation.

Length-tension diagrams of isometrically contracted muscles (curves of isometric maxima) are usually considered to be reversible curves, i.e., it is implied that tension follows this curve when length is altered sufficiently slowly. Release diagrams of isometrically contracted fibres show considerably lower values of tension at a given length than are indicated by the corresponding curves of isometric maxima, in spite of complete readjustment after release (6), while diagrams of fibres stretched during isometric contraction correspond approximately to the curves of isometric maxima. The irreversibility is due to a plastic increase in length which is irreversible during contraction (yielding). In terms of minute structure this is explained by the inhibiting effect



of tension on the propagation of a chain reaction in the contractile elements. The process of contraction stops when tension approaches a critical value, or it cannot start when initial tension exceeds a certain value, i.e., yielding decreases the degree of contraction in the minute structural elements. It is in agreement with this interpretation that the exponential expression (7) for the time course of extra-tension during isometric twitches fits experimental results as long as tension is moderate, while the time course of tension in tetanic contraction, or when twitches are released at high initial tension, cannot be explained in this way.

The residual contraction after tetanic contractions of moderate duration may amount to 50 per cent of the maximal extra-tension and is reduced by potassium cyanide (8).

*Thermoelasticity.*—At equilibrium length temperature is without influence on the tension of a resting fibre. At slight elongations (20 per cent) tension increases with increasing temperature, i.e., internal energy decreases, the linear coefficient of thermal expansion being negative. On further elongation a normal positive coefficient is noticeable and in whole muscle it passes zero (inversion point) at 45 per cent of stretch (9). In the isolated fibre an inversion point, if present at all, does not occur before the fibre has been stretched approximately 100 per cent (10). This difference is probably due to the presence of connective tissue in whole muscle with a positive coefficient of thermal expansion dominating at high elongation.

The length-tension diagram of myosin threads corresponds closely to that of the isometrically contracted fibre (11), but before this finding is taken as an indication that myosin threads contain myosin in a "contracted" state, it must be borne in mind that the S-shape of length-tension diagrams for myosin threads, like that in the case of rubber, may be due to a lower degree of initial orientation. Though Dubuisson (11) claims a total orientation of myosin threads at equilibrium length, the above interpretation is supported by the low values of specific birefringence found in myosin as compared with muscle (12).

*Elasticity, viscosity, and working capacity.*—The elastic properties of the isolated fibre were determined by measuring the increase in tension caused by small periodic variations in length with frequencies between 0.5 to 200 cycles per second (10, 13). Stiffness at rest and during contraction varies proportionally with



tension, and stiffness during contraction is up to two to three times higher than stiffness at rest, when referred to the same tension. The difference increases with increasing frequency of periodic length alterations, and decreases with increasing temperature. In this connection it is important to note that the structural changes in the contractile substance occurring during contraction are only characterised by that increase in stiffness which is not due to the increase in tension. As is the case in whole muscle, viscosity increases in the isolated fibre during contraction (6). The course of tension following sudden stretch and release (stress-relaxation curves) gives a time "constant" (velocity constant) which increases during the relaxation period. The interpretation of these results from experiments on isolated fibres leads to a conclusion similar to that obtained from recent work on whole muscle (14, 15, 16), viz., that a simple mechanical equivalent explaining the mechanical properties, especially their velocity relations and their temperature dependence, is difficult to provide. Viscous properties are considered as being due to the velocity of transition from one linkage modification in the minute structural elements of the contractile substance to another.

Stiffness measured with rapid variations in length corresponds to the differential quotient of length-tension diagrams registered at shortening velocities which are proportional to the frequency of these imposed variations in length (13). It is, therefore, possible to express factors which determine tension and work in a fibre by

$$y = (e^{Fz} - 1)b,$$

$$\text{where } y = \text{tension}, F = \frac{d \text{ (tension)}}{d \text{ (stiffness)}}, b = \frac{\text{initial stiffness}}{F} \quad (\text{identical at}$$

rest and contraction) and  $x$  = shortening capacity, i.e., the dynamic shortening of which a fibre is capable, when released to zero tension. The work performed by the fibre when released is obtained by integration of the equation with respect to  $x$  and can be determined without performing an actual release, e.g., at any moment during an isometric twitch.

Force-velocity relations arrived at from work on whole muscle assume a relatively simple dependence between shortening velocity and load, work becoming zero when a certain critical

critical velocity is applied (17, 18), e.g., following Ramsey's velocity constant  $\beta$ . In a mechanical model this would correspond to the presence of a shunt viscosity across the contractile substance. From stiffness and release diagrams of isolated fibres a corresponding relation cannot be expected, as  $\beta$  increases during the time of relaxation. Investigations of stiffness with different frequencies indicate a decrease in tension with increasing shortening velocity, but this decrease does not change in the same ratio as the increase in shortening velocity. This is supported by work diagrams registered at different shortening velocities of isometrically contracted fibres (19) as determined by Hill (16) for total muscle.

*Mechanical properties in relation to microscopic structure.*—Length-tension diagrams of the anisotropic (A) and isotropic (I) substances provide evidence for an active participation of the latter in the development of tension during contraction. Tension is caused by an increase in stiffness in I, while in A it is furthermore due to a reduction in the equilibrium length (6). From specific ultraviolet absorption curves it can be seen that adenine derivatives in resting muscle are preferentially localised in the I substance (20), where microincineration reveals the highest ash content (21). In fatigued muscle the sharp difference between strongly and weakly absorbing bands is more or less smudged out, and it is deduced from these findings that adenine derivatives in I in the course of muscle activity migrate to the A segment and that the major part of the phosphagen is situated in the I substance.

A method of recording changes in cross striations during contractions of the isolated fibre is described, but results of its application are not yet given (22). By microcinematographical registration variations in cross striation and tension are registered during contraction (23). Tension during contraction is delayed in proportion to changes in cross striation (24). In contractions released from low and moderate lengths changes in cross striation are independent of the strength of direct stimuli and the gradation in tension hereby produced. Not unless stretch reaches 80 per cent is gradation detectable in the variations of cross striation. These findings are quantitatively explained by the presence of non-contracted substance side by side with contracted in the sub-maximally contracted compartments of a fibre, and it is concluded that gradation in fibre tension as produced by stimuli of different

strengths is due to a variation in the number of active elements and not to a gradation in the individual minute structural elements. Thus for a given elongation, there exists an "all or none" relation for these elements. Even if uniform changes in cross striation are found in the entire fibre, the conclusion cannot be maintained (23) that contraction is propagated without decrement, since variations in cross striation at low and moderate degrees of stretch are unsuitable indicators of the degree of contraction. It is, however, worth mentioning that in contrast to findings in nerve fibres, there exists a combination of gradation and propagation in the muscle fibre. The rate of propagation over the fibre of changes in cross striation is 0.3 m. per sec. (at 20° C.), i.e., essentially lower than that of the spread of the action potential. The relatively low propagation velocity is of decisive influence on the initial course of tension and thereby on the latency period. Even assuming that there is no latency period at all, a measurable development of tension would not be detectable before approximately 1 msec. had elapsed in a fibre of only few millimeters length and with a registration sensitivity of 0.001 per cent of maximum tension. The equation given by Gilson *et al.* (7) gives an adequate expression also for the course of tension in the initial stage of contraction, but when evaluating the factors determining this part of tension, attention must be paid to the finite rate of propagation and the consequent passive stretch of elastic elements before activation, which alone may explain the increasing differential quotient of initial tension. The initial relaxation occurring in whole muscle during the latency period of contraction has been analysed in detail by means of a highly sensitive piezoelectric myograph (25, 26, 27). The latency relaxation is reduced in amplitude by previous activity and varies with stretch. Its duration is shortened with increasing values of pH. Increasing strength of stimulation enhances the degree of initial relaxation, though the subsequently developed contraction tension has almost reached constant values. Mainly on the basis of the pH and temperature dependence the latency relaxation is interpreted as the mechanical sign of a tension-induction process involving the mechanochemical coupling of myosin and adenosine-triphosphate. The latency relaxation has, however, also been interpreted as a mechanical wave phenomenon, e.g., on the basis of model experiments on water-filled rubber tubes (28). It seems

probable, though it is still unproved, that similar conditions for the propagation of mechanical waves can exist in total muscle. Apart from experiments with initiation of stimuli in different regions of the muscle and model experiments on rubber tubes, the recognition of the latency relaxation as being due to a compression wave would require initiation of a similar wave phenomenon in resting muscle. It may be of interest to mention that tension in isolated short fibres develops 1 msec. after stimulation, i.e., at a moment when the latency relaxation starts in whole muscle. In view of these different interpretations it seems preferable to wait for further experimental evidence concerning the correlation between chemical processes and latency relaxation before discussing equations with several more or less approximate parameters (29).

After stimulation, within a period where extra-tension is slight, often so slight that it is difficult to decide whether tension is present or not, considerable changes occur in the fibre stiffness which are inherent in the contraction process itself (13).

The time course of the relative length of different segments in whole muscle varies during contraction (30). As may be expected, in regions where the cross section is small or the stimulation intensity relatively low, stretch will occur during contraction, other segments will really shorten, and in others the length will remain unchanged. Segments once stretched in contraction will not shorten further. No explanation is given for this behavior, which on the basis of experiments on isolated fibres (6) might be interpreted as being due to yielding, inhibiting a subsequent shortening (elastic locking).

*Cardiac muscle.*—The theory of a helicoidal arrangement of cross striation has recently been revived (31), though without providing new convincing evidence. Length-tension diagrams of small bundles of cardiac muscle fibres are S-shaped, indicating a lower degree of preorientation than found in skeletal muscle, and maximal tension during contraction is attained at a considerably higher initial tension than in the latter. Young's modulus ( $0.1 \times 10^{-6}$  dynes $\times$ cm. $^{-2}$ , static) is one fifth of the value found in skeletal muscle. The difference in extensibility of A and I is the reverse of that in skeletal muscle, while stiffness in both cases varies proportionally with tension. Stiffness in cardiac muscle fibres is, however, not increased during contraction when referred to the same

tension as at rest, not even at low temperatures. Viscosity dominates the mechanical properties of cardiac muscle to a much higher degree than is the case in skeletal muscle (32).

#### ANALYSIS OF MINUTE STRUCTURE

Analysis of minute structure in its relation to function is one of the most important tools in investigating the process of muscular contraction. The methods that have come into use are partly indirect, such as determination of birefringence and other optical properties, thermoelasticity and elasticity, and partly direct, such as x-ray diffraction and electron-microscopical observation. Indirect investigations have been dealt with in the section on mechanical properties; work on birefringence has been rather scarce and is discussed in the section on adenosinetriphosphate and muscle.

*X-ray diffraction and electron microscopy.*—At large angle diffraction, a well-developed pattern has been obtained from a one-year-old smooth muscle preparation. It is interpreted as being of the monoclinic type with a twofold screw axis (space group  $C_2^2$ ). As freshly dried preparations show considerably fewer details, it is open to question whether this pattern is a true expression of muscle structure or the result of the process of drying (33). Comparison between x-ray patterns of wet and dried muscles indicates that only a small amount of water is located intramolecularly. In isotonic contraction (time of exposure, six minutes) orientation disappears, but is restored when the contracted muscle is brought under isometric conditions. No information is provided as to whether the pattern of an isometrically contracted muscle differs from that of the resting muscle (34, 35). By the use of small angle diffraction derived from the larger periodicities of the structural elements most interesting results have been obtained. A common fibrous element present in both smooth and striated muscle could be identified with a period of 350 to 420 Å along the fibre axis (36, 37, 38).

Electron-microscopical observation of unstained myofibrils from striated muscle fibres (39, 40, 41, 42) reveals transverse bands which correspond to the cross striations visible in the usual light microscope. Fibrillar structures of 50 to 100 Å in diameter have been observed in specially thin sections of striated muscle (43). In smooth muscle (clam adductor) a regular structure with a

period of 1100 Å is found, but there is no segmentation corresponding to that present in the cross striated muscle fibre. The fibrils are shown to pass continuously through both the A and the I substance of the striated fibre, and it is assumed that the higher density in A is caused by the presence of some other substance which might be closely associated with the fibrils in the A substance (39). Previous minute structural analysis indicated, however, that differences in the orientation of the main axes of the molecules in the two substances could be responsible for the differences in refraction (24). No coiling or folding of filaments is seen in isotonically contracted fibrils and shortening must be localised in molecular elements within the contractile filaments which are not resolved by the electron microscope (39). Although these conclusions also appear highly probable in view of former more indirect structural analysis, it will be necessary to regard with some reserve results which concern changes in cytological structures produced by contraction, since electron-microscopical observation is restricted to fixed and dehydrated material, and since it is difficult to fix a fibre in the contracted state. In electron micrographs the Z membrane appears to be of amorphous material which "glues" the filaments together. The basal membrane apparently passes transversely through a fibril, and it can be conclusively stated that the Z membrane does not consist of collagenous tissue, as was suggested by Häggqvist (44). Myosin filaments prepared from skeletal muscle or smooth muscle show on electron-microscopical examination fibrils of about 50 to 250 Å in diameter and of highly variable length, extending up to several microns (45, 39). From smooth muscle (molluscan muscle) a protein different from myosin (insoluble in potassium chloride and disintegrated by Edsall's solution) has been obtained which after staining reveals a well-defined structure. The axial periodicity of this paramyosin is 725 Å (40).

#### ADENOSINETRIPHOSPHATE (ATP) AND MUSCLE

In the last years most promising advances have been made in establishing a close correlation between structural proteins and chemical processes in muscle. The stimulus to this new development was the discovery that myosin acts as an adenosinetriphosphatase (46) and that ATP produces striking changes in the physical properties of myosin solutions (47). Although the identity

of myosin with adenosinetriphosphatase (48) has been questioned (49), it was only quite recently that a native myosin free from adenosinetriphosphatase activity could be prepared (50, 51) and the nonidentity of myosin with adenosinetriphosphatase proved (52). The essential nature of the protein sulfhydryl groups in this enzyme activity has been demonstrated by the use of sulfhydryl reagents and by the reversible transformation of the sol group into the disulfide form (53, 54). Szent-Györgyi and his school (55) have investigated the behavior of muscle proteins especially towards ATP. It was found that myosin can be extracted from muscle in two forms, viz., myosin proper and actomyosin. The latter consists of two compounds, directly extractable myosin and actin, which latter is obtained by previous treatment of the muscle with defatting solvents. Threads from actomyosin show a considerable volume constriction upon addition of ATP, this effect being highly specific. It is, however, still open to question how far this "contraction" *in vitro* is a true model of the contraction process. Thus, the changes in flow birefringence and viscosity produced by ATP in solutions of actomyosin are found to be due to a disaggregation of the actomyosin micelles and do not correspond directly to the primary mechanism of contraction (56). The quantitative study of the effect of ATP on several physical properties of myosin and actomyosin showed that myosin acts in units of particle weight  $10^4$  (57, 58). Tropomyosin, a fibrous protein showing flow birefringence, has recently been prepared from muscle by methods similar to those used for the preparation of actin; it is, however, not identical with the latter (59). An interesting attempt has been made to explain the shortening of the myosin molecule by analogy with experiments on amino acids (60), the phenomenon being said to be due to the interaction of a sulfhydryl and a phosphorylated hydroxyl side chain under the influence of adenosinediphosphate (ADP). A thioether linkage and ATP would thereby be produced. Relaxation would involve the phosphorylytic cleavage of the thioether linkage by ATP (61).

ATP or ADP in minute amounts ( $10^{-8}$   $\mu$ g.), applied to the curarised or noncurarised isolated muscle fibre of the frog, releases tetanus-like contractions which are accompanied by action potentials (62). Also in curarised and noncurarised, striated and smooth muscles of mammals contractions are released when the substance is applied by close arterial injection (63, 64). In the



isolated fibre, birefringence decreases reversibly after application of ATP and ADP and, by virtue of its time course, is interpreted as an expression of restitutional processes on the contractile protein. Whereas release of contraction is also brought about by inorganic triphosphate or inosine triphosphate, the measured changes in birefringence are specific for ATP and ADP (62, 65). Creatine-phosphate and acetylphosphate are without effect on striated muscle, thus indicating once more that the breakdown of ATP is the reaction nearest in time to the physical process of contraction (65). Application of ATP to a muscle increases its sensitivity not only to subsequent doses of acetylcholine (63, 66, 67), but also to electrical stimulation in curarised muscle.

ATP's role as a shock producing factor in ischaemic shock (68) has been questioned, as ATP is destroyed in muscle during occlusion (69, 70); it is, however, claimed that damaged muscle is able to form, but unable to store ATP, which, therefore, cannot be ruled out as a possible factor in shock (71). A toxic factor in muscle extracts was found by analysis of the ash to be potassium. This may have an effect when extracts are used to produce shock (72).

In view of the central position of ATP in muscle physiology it is of importance that the structure originally ascribed to it by Lohmann (73) has been proved conclusively (74). The yield of ATP from rabbits under magnesium anesthesia is greatly increased (75). Whether this is due to a specific effect of magnesium on the breakdown of ATP or to its paralysing action is undecided. With regard to studies of the metabolism of ATP and other phosphate compounds in muscle, the reader is referred to a recent review of the chemistry and metabolism of phosphorus compounds (76).

#### PERMEABILITY AND ELECTROLYTES

Boyle & Conway (77) interpret their experiments on frog's sartorius in isotonic and hypertonic solutions with different potassium concentrations by assuming permeability for chloride as well as for potassium, but not for sodium ions. Calculation of the permeability constants reveals the highest values for potassium chloride (78). The application of these results to the physiological permeability of the muscle fibre has been questioned, as a potassium concentration of 10 mM will already create unphysiological conditions (79, 80, 81), and as irregularities occur at these low physiological concentrations in the constant equilibrium otherwise



found between potassium inside the fibre and the surrounding isosmotic solutions with different potassium contents. According to Krogh (80), who gives a detailed review of work on permeability with special emphasis on investigations with radioactive tracers, an active elimination of sodium with simple diffusion of potassium would account for the changes observed. In a later paper, Conway (82) points out that a sodium transport of the same magnitude as the potassium permeation would demand large quantities of energy for which it may be difficult to account.

Potassium inside the fibre occurs in a concentration thirty to forty times that of the surrounding fluid and must be assumed to be largely free to establish the osmotic pressure. During contraction, ion permeability increases, potassium being lost and sodium entering the fibre. Normal conditions are restored during recovery, potassium being regained and sodium lost, both against the gradient (83). Magnesium is likewise shown to enter the fibre against the gradient (84). It is rather puzzling that a large fraction of the potassium (60 per cent) in experiments with  $K^{42}$  is found to be practically nonexchangeable (85). No satisfying explanation can be given for these observations, since we are forced to assume some internal structure which prevents the free exchange of potassium ions. In contradiction to these findings, Fenn *et al.* (86, 87) find in their experiments a more than complete interchange between potassium in muscle and plasma. They explain the former results by assuming the presence of minute amounts of  $Na^{24}$  in the radioactive potassium injected. The slightly higher values of  $K^{42}$  in muscle, compared with those in plasma, might be due to the fact that in the last mentioned investigation the specific activity of muscle is compared with  $K^{42}$  in plasma measured at the end of the experiment and not with a mean value for radioactive plasma potassium determined during the experiment (88).

On the other hand, in investigations in which the whole animal (rat or rabbit) was dissolved after the experiment and the specific activity of the plasma  $K^{42}$  compared with that of the total animal, a full interchange was only found in the case of the rat, while in the rabbit only 50 per cent of the radioactive potassium was exchanged. As approximately two thirds of the potassium is concentrated in the muscles, and as also in the rabbit a complete interchange between muscle and plasma potassium is claimed to be present, the partial exchange found in the whole rabbit still awaits an explana-

tion. A general objection to these experiments with labelled potassium is the necessity of introducing appreciable amounts of potassium into the circulation, under which circumstances an effect on the normal cell permeability can hardly be excluded.

Investigations on heart muscle (89) seem likewise to indicate a restricted exchange of  $K^{42}$ ; the potassium-calcium balance essential in contraction is shown to refer to ions which are present in the fibre surface.

A diffusion of sodium into the muscle cell in relatively higher amounts than chloride, when the concentration of a surrounding sodium chloride solution is increased, is also indicated in Steinbach's experiments (90). The quantitative application of these results will be affected by the rather large standard deviation in the determination of tissue sodium and potassium. Since muscle is shown to behave like a simple osmotic system with respect to volume and weight changes and yet shows changes in ion permeability which are not in agreement with this conception, one is drawn to the conclusion that permeability cannot be fully explained in terms of a semipermeable membrane with definite permeability. It scarcely appears justifiable to assume a large fraction of osmotically inactive space on the basis of these experiments, since Hill & Kupalov (91) and Boyle & Conway (77) have convincingly shown that practically all the ions in the fibre are necessary for osmotic equilibrium. Osmotic equilibrium cannot be expected at the same concentration of cations on both sides of the membrane. A considerable part of the anions inside the fibre have more than one valency and may bind a larger proportion of the alkali ions.

By comparison of the distribution of inulin and chloride, the amount of the latter in the fibre is estimated and the uncertainties in the determination of extracellular space are discussed (92). Results regarding the penetration of ammonia into frog muscle are rather conflicting. At constant pH and constant bicarbonate content the ammonia taken up is found to equal the potassium and sodium lost, and after five hours the concentration of ammonia per gram of muscle is the same as the concentration in the solution (93). Conway & Moore (94) object to these results and claim that part of the fibres irreversibly lose their differential permeability for potassium and sodium. They consider the undamaged fibre as being practically impermeable to ammonium. An increase in carbon

dioxide is, however, found to facilitate the inward diffusion of ammonia.

Verzár gives an interesting review of the history of theories of contraction. On the basis of an attempt to correlate potassium with both the carbohydrate metabolism and the contractile protein, he develops his own theory of contraction. It implies the hypothetical assumption that myosin is enzymically active only in the contracted state (95). The finding of an inhibition of potassium-liberation in the muscular contraction of adrenal-deficient rats, which provided an argument in favour of the connection between carbohydrate metabolism and potassium, could not be reproduced in recent experiments (96).

#### NEUROMUSCULAR TRANSMISSION

The amount of acetylcholine in tissue cultures of cardiac muscle depends upon the presence of nervous tissue (97). In fatigued striated muscle the acetylcholine concentration decreases and choline esterase activity increases (98). A remarkable dissociation has, however, been found in the effect of acetylcholine on the one hand and electrical stimulation on the other. Stretch reduces the effect of chemical stimulation without affecting electrical excitability. In a muscle fatigued by electrical stimulation acetylcholine is still highly effective (99). Atropine has a blocking action on contractions produced by acetylcholine, while electrical excitability is claimed to persist (99, 100, 101). In the evaluation of these results it is, however, necessary to bear in mind that the application of acetylcholine by close arterial injection, or directly to the end plate, implies the passage through other interfaces than are involved in the liberation of a possible chemical mediator by electrical stimulation.

On the isolated phrenic nerve-diaphragm preparation of the rat, it was found that epinephrine affects the muscular response to nerve stimulation, independently of the vasoconstrictor action of the drug (102). It is, however, rather puzzling that in nonfatigued muscle only submaximal stimulation of the nerve results in an increase of the mechanical response after addition of epinephrine to the surrounding fluid. Prostigmin and physostigmin augment this effect which is attributed to an improvement of neuromuscular transmission. No explanation is offered for the differences between submaximal and maximal stimulation. Apart from the rather

remote possibility that contraction of muscle fibres from one motor unit during submaximal stimulation might induce contraction in neighbouring fibres from other units, the lack of epinephrine effect during maximal stimulation might be due to an incomplete proportionality between the mechanical response and the active fibre mass. In recording with a light isotonic lever, the degree of shortening as a function of the number of activated fibres will vary the less the more fibres are activated. Procaine (103) and atropine (102) besides acting like curarine by raising the threshold due to the extermination of end plate excitability, also diminish the liberation of acetylcholine from the motor nerve endings.

Hitherto, most observations have indicated that the liberation of the chemical mediator was localised to the nerve ending in the motor end plate. Coppée (104) assumes it to be liberated in the sole, i.e., in the myogenic part of the end plate. Except in some muscles of the cat, however, it is believed of subordinate importance in neuromuscular transmission of normal muscle, and the potential set up at the end plate is used as an indicator of electrical transmission. In formulating an electrical theory of transmission the action of curare is explained by the depression of the junctional potential (105). This effect, which had previously been observed on isolated end plate-muscle fibre preparations in the lizard (106), was recently affirmed in work on the isolated end plate-muscle fibre preparation of the frog (107). Combining certain aspects of a chemical and an electrical theory of neuromuscular transmission, the action of acetylcholine is interpreted as a depolarizing effect which is specifically localised to the region of the nerve ending, there giving rise to a local potential which may set up a propagated response (107a). From experiments on curarised fibres, the rather surprising result is obtained that there is no evidence for the existence of a specific excitability of the end plate (108). Since, however, the blockade of the myogenic part of the motor end plate demands rather high concentrations of curarine (more than four times the concentration necessary for neuromuscular blockade), it will be difficult to exclude the possibility that the identity in excitability of the nerve ending and other parts of the muscle fibre may be due to incomplete curarisation. Experimental tetany is characterised by repetitive impulses set up by a single nerve volley in the motor end plate (109). A similar reaction, which has previously (110) been described for the effect of experimental tetanus, could not be ob-

served in recent experiments (111). Immobilisation of a muscle in a short length, as also brought about by tenotomy, produces changes in the neuromuscular transmission which are explained as being due to a reflex mechanism initiated by the abnormal tension (112). Neuromuscular transmission in certain muscles is facilitated by a decrease in pH. This effect, which is confined to respiratory muscles, is interpreted as being caused by the inhibition of choline esterase. It remains, however, an open question, why previous treatment with acids in other muscles has the opposite effect, viz., reducing the action of acetylcholine. Increased tension of carbon dioxide with constant pH of the surrounding fluid causes an improvement in neuromuscular transmission also in locomotor muscles. This finding is explained by assuming an acidification of the cell content and conclusions are drawn as to the existence of a more general mechanism of acid humoral intermediation (113, 114, 115). The correlation between acetylcholine content and electromotive force (expressed in volts per centimeter) in the electric organ of the electric fish is an essential argument in a theory which tries to explain neuromuscular transmission and the propagation of the impulse in the nerve fibre (116, 117, 118, 119). Estimation of the acetylcholine content is based on the determination of choline esterase. The electrical energy from the discharge of the electric organ is approximately one fourth of the energy that can be accounted for by the breakdown of creatinephosphate, and an estimate of total energy from experiments with discharges over different shunt resistors across the organ gives agreement between energy supplied from creatinephosphate and electrical energy (120). It must, however, be borne in mind that all evidence for this theory of neuromuscular transmission is built on the assumption of a parallelism between acetylcholine metabolism and cholinesterase concentration. For a detailed discussion of this question the reader is referred to a recent review by Feldberg (121). An important factor in the enzymic synthesis of acetylcholine by acetylase is adenosinetriphosphate which under certain conditions can be replaced by citric acid (122, 123, 124, 125). As to the form in which acetylcholine is present in muscle no definite statement can as yet be made. The fact that extracts can be prepared from muscle which on heating or acidifying show an increased amount of acetylcholine (126, 127, 128) has recently been affirmed by Abdon who in several papers has considered this problem in more detail (129, 130, 131,

132) [see also (133)]. He showed that on alcoholic extraction of frozen tissue acetylcholine could be extracted in an inactive form, from which it was liberated by heat or acid. Acetylcholine formed at stimulation is resynthesised to this labile complex compound, and as the resynthesis is inhibited by iodoacetic acid, it is concluded that the necessary energy is derived from glycolysis. In contrast to former investigations (134) no free acetylcholine is found when the nerve of a curarised muscle is stimulated, and from the comparison of indirect stimulation of curarised and non-curarised muscles it is concluded that the labile complex compound in the former is not broken down as is the case in normal muscles (130). However, attention must be paid to the fact that these experiments imply a comparison between contracting and non-contracting muscles. Since the inactive acetylcholine compound is found to be evenly distributed over the muscle fibre, it is thought to be associated with the process of muscular contraction (130a). Before accepting this explanation, it will be necessary to compare quantitatively the amounts of acetylcholine liberated at motor end plates with those found in the fibre. Ether and chloroform in low concentration increase and in higher concentration decrease the sensitivity to acetylcholine in the rectus abdominis muscle of the frog; with the concentrations investigated, contractions evoked by potassium are not affected by these drugs (135). It does not seem quite evident how far the systematic application of a large number of cyclic compounds to striated muscle broadens our present knowledge of the different mechanisms involved in potassium and acetylcholine sensitivity (136).

#### MYASTHENIA

The neuromuscular block in frog's muscle, caused by serum from patients with myasthenia gravis (137), can either be due to a curare-like action of this serum or to an inhibition of the acetylcholine synthesis. Since application of acetylcholine has identical effects on muscles kept in normal and in "myasthenic" serum, it is assumed that the acetylcholine synthesis is depressed in myasthenia (138). In these experiments the "myasthenic" serum was, however, kept for more than six hours before being tested on the rectus femoris muscle of the frog, and as a possible blocking factor would already be inactivated, no decisive conclusions can be drawn from these experiments. On the other hand the observations

of Wilson & Stoner (137) could not be affirmed, serum from normal and from myasthenic fatigued muscles having identical effects on the electrical excitability and on the contractility of gastrocnemius or semitendinosus muscle from a frog (139). After passive and active movements of normal muscle a curare-like factor is found, the blocking action of which is claimed to be highest after passive movements (140). Serum from an immobilised normal extremity added to brain debris exhibits a larger amount of resynthesised acetylcholine than "myasthenic" serum taken under the same conditions. This difference disappears when serum from fatigued muscles is applied (141).

Muscular exercise of undefined intensity causes no change in the choline esterase content of serum in normal individuals or in patients with myasthenia gravis (142); in another investigation (143) a statistically significant increase in choline esterase is found after exercise of 830 to 1100 kgm. per min. The increase in serum choline esterase is accompanied by a decrease of this enzyme in the blood corpuscles. Within the limits of an acute experiment, double adrenalectomy does not reproduce the syndrome of myasthenia gravis (144). The experimental basis for the curative effect of the extirpation of the thymus appears rather uncertain (145).

#### ELECTRICAL ACTIVITY

The potential distribution along the surface of an indirectly stimulated skeletal muscle shows no sign of a progressive wave of electrical involvement from one end of the muscle to the other, such as is found in directly stimulated curarised muscle. As previously described for heart muscle, action potentials in skeletal muscle have a polar distribution, regions of positive and negative polarity arising simultaneously. Movement of potential maxima occurs during only part of the action potential period (146). The injury potential of living frog's sartorius muscle increases with moderate elongation. This effect is only partly reversible and is interpreted as a diminution of the true polarisation potential (147). The negative after-potential of the isolated nerve-muscle fibre preparation is increased by veratrine, and if this potential reaches a critical value, repetitive impulses are initiated. Veratrine in the concentrations investigated does not affect the resting potential of the uninjured fibre, nor does it alter its acetylcholine or potassium sensitivity (148). Electrical stimulation of the motor area of



the cortex in rabbits results in action potentials in the muscles of the foreleg which are synchronous with the stimulation frequency as long as this does not exceed 10 cycles per second. Electrical and chemical stimuli release a pronounced after activity of central origin. After central application of strychnine the maximal frequency of impulses from the single motor unit amounts to 125 per second (149). Evidence is obtained that the small fibres in ventral roots previously assumed to constitute a separate motor system in the service of muscle tone (150), release no motor activity and most probably transmit sensory impulses originated in muscle (151). Spontaneous "doubling" in the rhythmic discharges in motor neurones from soleus and triceps muscle are interpreted as being due to a supernormal period in the recovery cycle of these neurones. This assumption is supported by the finding of an increased "doubling," when procedures are applied which enhance supernormality in peripheral nerves (152). Doubling, tripling and polyphasic action currents from single motor units are often met with when leading off during voluntary contractions of muscles in man; they are interpreted as being due to an asynchronous activity of different fibres from the same motor unit which is caused by differences in the propagation rate of the impulses. The rate of propagation is thus found to vary between 1.5 and 12.5 m. per sec. (153). As we may assume that differences in the times of arrival of the nervous impulse to the motor nerve endings in different fibres of the same unit are extremely small, and as the position of the electrode relative to the starting point of the propagated impulse is fortuitous, no proportionality can be expected between the time differences in the action potentials and the distance between the different leading-off electrodes. A determination of the rate of propagation would imply leading off from the same fibre with the electrodes situated on the same side of the starting point of the propagated impulse. In volitional contraction of normal human muscles, motor units are claimed to react with a constant frequency of 6 to 14 impulses per second, irrespective of the strength of contraction (154). No attempt is made to explain the well-established contradictory findings which are especially convincing in the case of partial lesion of the motor nerve.

Resting potential and rectification in sartorius muscle of frogs are decreased by an increase of potassium in the surrounding fluid. The difference in potassium concentration inside and outside the



cell will cause different electrolyte contents of the membrane in different phases of alternating current flow. With increased potassium concentration in the surrounding fluid rectification and resting potential decrease with conductivity on both sides of the membrane and approach the same value for both directions of the alternating current (155).

#### ELECTROMYOGRAPHY

Motor unit action potentials from voluntarily innervated muscles are most commonly mono- or diphasic, but tri- and polyphasic action potentials are observed in all voluntary muscles (156, 157, 153). In denervated muscle, fibrillation gives rise to a characteristic action potential of extremely short duration generated by the activity of a single fibre, while fasciculation is due to the activity of many fibres in a motor unit (158). Fibrillation occurs a few weeks after denervation and lasts until reinnervation occurs; it is not related to the degree of atrophy. Fibrillation is considered an indication of a lesion somewhere in the lower neurone and the appearance of motor unit potentials of normal duration (5 to 10 msec.) is an early sign of recovery after denervation. Before these potentials are detectable a significant decrease in the number of fibrillation action potentials is recorded (157, 159). In genuine muscular atrophy maximal contraction generally is accompanied by an interfering electrical activity of many motor units. Since even slight contractions demand a larger spread of innervation than in normal muscles, it is here more difficult to produce "single discharges" than in normal muscles. In neurogenic atrophy the number of active motor units is markedly decreased and during maximal effort single discharges are found without interference from neighbouring fibres (156). By leading off simultaneously from different regions of the same muscle, i.e., from different motor units, a further differentiation of atrophy is facilitated, characterised by a synchronous activity of different motor units in cases of neurogenic atrophy of intramedullary origin. In normal muscle, as in muscle with neurogenic atrophy caused by diseases of the nerve roots or the peripheral nerves, synchronous activity may occur as an exceptional transient phenomenon (160). In poliomyelitis synchronisation may appear very soon after the onset of the paralysis. Comparison of different types of innervation (synchronous, partly synchronous, and

asynchronous activity) with the clinical course in cases of poliomyelitis indicates that the chance of recovery is most favorable in cases of continuous asynchronous activity. Synchronisation is presumably caused by the irradiation of impulses over a larger range of ganglionic cells or by the retained activity of ganglionic cells with high synaptic concentration. While in normal or atrophic muscles, when atrophy is of peripheral origin, fatigue is accompanied by falling out of action potentials without any decrease in their amplitude; in stationary pareses after poliomyelitis, fatigue is accompanied by a gradual decrease in the action potentials of the single fibres (161). Previous findings indicated a widespread hyperirritability to stretch accompanied by electrical discharges in apparently resting parietic muscles in acute poliomyelitis (162). More recent investigations have revealed little or no electrical activity in resting muscles in the acute stage of the disease (163, 161). In later stages (about the third month) Watkins *et al.* (163, 164) find spontaneous electrical discharges of high voltage in partially paralysed muscles. Spasticity and rigidity are both considered to be peripherally induced variations of normal postural reflex activity, due to the release from extrapyramidal inhibition. In rigidity the electromyogram furthermore shows disorders in innervation in the form of rhythmic discharges at slight innervation. The pattern of these rhythmic discharges can easily be imitated in volitionally released tremor and is therefore insufficient to differentiate between hysterically and organically produced tremor (165).

#### DENERVATION AND ATROPHY

Close arterial injection of curarine releases contraction in denervated muscles of dogs (166). Similar effects have not been observed in frog or cat muscles. Therefore, although curarine also depresses the effect of acetylcholine in denervated muscle, it seems rather doubtful how justifiable it is to consider these results as significant of a purely myotropic action of curarine. It is, on the other hand, well established that acetylcholine has a curarising action on normal and denervated muscle (167). The stimulating action of adenosinetriphosphate in denervated muscle of the cat is abolished by previous application of acetylcholine (167).

Loss of tension occurs in denervated muscles at a more rapid rate and to a greater extent than the weight loss, while conversely,

tension returns more rapidly on reinnervation than the increase in weight (168). In atrophy due to denervation, the protein concentration of muscle is reduced to a much higher degree than in atrophy caused by immobilisation. Atrophy due to tenotomy, however, gives the same changes in weight and protein concentration as that due to denervation (169). Myosin extracted from denervated or immobilised muscles has a diminished solubility and a tendency to form much denser precipitates than myosin from normal muscle. It is less able to be spun into threads, and threads from atrophic muscles have lower values of birefringence than myosin threads from normal muscle (170, 171, 172). From experiments with artificial stimulation and disuse, atrophy caused by tenotomy is interpreted as being due to the extremely shortened state of the muscle fibres and not to the inactivity as such. The shorter the muscle during contraction, the less efficient daily muscular stimulation tends to become (173). On the other hand, spontaneous functional recovery is considerably better in muscles which are immobilised in minimal length than in those which are immobilised in maximal length (174). Strength-duration curves from patients with nerve injuries reveal an apparently significant displacement of the curves relative to those of normal muscle. On reinnervation improvement of excitability appears a short time before the earliest sign of recovery detectable by purely clinical means (175). From experiments on denervated muscles of dogs it is, however, concluded that strength-duration curves are not suitable for a quantitative study of changes in excitability of the neuromuscular system during the recovery period of reinnervation (176). The optimum frequency for stimulation of denervated muscle by alternating current is considerably lower (2 to 3 cycles per second) than in normal muscle (60 to 100 cycles per second), while in the former the optimum frequency increases with loading and rises up to 25 cycles per second (177). The increase in force observed in the initial stage of recovery after partial denervation is due partly to a hypertrophy of the residual normal motor units (178), and partly to the additional ramification of motor nerves from intact segments which take over the supply of denervated fibres. The latter conclusion is supported by the finding that the denervated portion of partially intact muscle atrophies to a lesser extent than would be the case if the muscle were completely denervated (179, 180). Regeneration of muscle tissue after

ischaemic necrosis of large portions of a muscle results from proliferation from pre-existing muscle fibres (181). In the rabbit the outgrowth proceeds at a rate of 1 mm. per day (181), while it may be mentioned for comparison that the rate of outgrowth of the tip of a severed nerve is as much as 3 mm. a day (182). With regard to the value of electrical treatment there is agreement that it can retard the rate of atrophy (weight loss), but cannot prevent the gradual loss of the mechanical response in denervated muscle. When electrical treatment has been applied to a muscle, its fibres appear larger and are said to be stronger at the time of initial reinnervation than they would have been without treatment. This initial advantage disappears, however, despite a continuous treatment during the later stages of regeneration (183, 168). Thus the value of electrotherapy would appear chiefly to consist in keeping the denervated muscles in a better condition until reinnervation begins. Stimulation applied to a loaded extremity is claimed to be more effective than in unloaded muscle and the deterioration of muscle proteins is retarded by electrical stimulation (184, 185). Atropine, which previously had been claimed to delay atrophy, is shown to be without specific effect. It influences neither the reduction in strength of contraction nor the changes in birefringence (186). In spite of the fact that war injuries have considerably stimulated experimental work in this field, the lack of systematic investigations as to the effect of the duration of the electrical treatment, the shape of the current pulses, the condition of the muscle, etc., is quite evident. However, detailed histological studies of the neuromuscular system during denervation and reinnervation in cases of peripheral nerve injuries have been performed (182, 187, 188, 189). A more extensive use of muscle biopsy in clinical diagnosis is recommended as a means for the differentiation of neurogenically or myogenically initiated muscle atrophy and for an estimation of the degree of atrophy (190). Ultraviolet microscopy in muscular dystrophy reveals, apart from the changes that also are visible in white light, variations in the appearance of the sarcolemma with areas showing intense absorption alternating with regions of slight absorption (191).

#### MISCELLANEOUS

The oxygen consumption of the extraocular muscles in guinea pigs is considerably higher than that of other muscles. This differ-

ence disappears in animals treated with thyroxine which thus affects the various muscles differently (192). Carbon monoxide may increase or decrease the oxygen consumption of frog muscle according to the intensity of the respiration (193). The oxidation of carbon monoxide to carbon dioxide cannot explain the after-effects of carbon monoxide which consist in an increase in the respiratory quotient (194).

The liberation of histamine accompanying muscular effort is larger than that following arterial occlusion of the same duration and is considered a factor which provides balance between the metabolic requirements of the tissues and their blood supply (195).

Different compounds of the vitamin B complex are found to increase the total work output of perfused gastrocnemius muscle in frogs (196, 197). Thiamine hydrochloride and especially thiamine pyrophosphate (cocarboxylase) have a considerable effect, while riboflavin and pantothenate do not affect the work output (198). No information is provided as to the localisation of this action in the muscle.

Intramuscular pressure is considered a measure of muscle tone (199, 200). Its decrease in shock, and its increase after sublethal hemorrhage without shock, are explained by an involvement of the somatic nervous system in the vascular adjustment to severe hemorrhage (201). The observation that changes in intramuscular pressure are unaffected by denervation in the anesthetised dog makes this interpretation rather doubtful (202).

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INSTITUTE OF NEUROPHYSIOLOGY  
COPENHAGEN, DENMARK

## EXERCISE<sup>1</sup>

BY PETER V. KARPOVICH<sup>2</sup>

*Robinson Foundation, Inc., New York and Springfield College,  
Springfield, Massachusetts*

This chapter will deal mainly with the effects of exercise upon organs and their functions, ergogenics, physical fitness, and exercise for convalescents.

The literature reviewed here shows the imprint of war on research related to the physiology and hygiene of exercise. For this reason, there is a strong emphasis on physical fitness, so obviously important from a military standpoint. Most of the articles and unpublished reports used in preparing this chapter are based on work done recently in this country. Some of the articles reporting experiments done abroad are several years old owing to disrupted communications during the war.

Very few physiological studies have come from schools of physical education—a logical place for such research. Most of these schools seem to be more interested in problems of organization and administration than in developing a scientific base for physical education. It is a sincere hope of this reviewer that this situation will be remedied soon.

### EFFECT OF EXERCISE UPON ORGANS AND THEIR FUNCTIONS

*Muscles and bones.*—The work capacity of muscles seems to depend merely on the size of the muscles, being 6 m. kg. per min. per sq. cm., and independent of age (13 to 48 years) (1). The increase in work capacity caused by training coincides with an increase in glycogen and creatine content of the muscles. The aerobic and anaerobic glycolyses are also augmented by training (2).

It has been an accepted axiom that exercise affects the structure of bones and ligaments. Yet, Lanier (3), in his experiments on mice, comes to an interesting conclusion that exercise has no important effect upon the formation of the tibial crest to which the patellar ligament is attached. Additional experiments on larger animals are probably desirable.

<sup>1</sup> This review covers the period from September, 1944 to August, 1946.

<sup>2</sup> Formerly: Chief, Laboratory of Physical Fitness, AAF School of Aviation Medicine, Randolph Field, Texas.

*Respiration.*—Control of respiration in general, and during muscular exercise in particular, has recently been the subject of lively discussion in physiologic literature. Gray (4) has succeeded in correlating the known data by a theory of multiple factor control. According to his theory of multiple factor control the amount of lung ventilation is determined by the algebraic sum of the partial effects of chemical, thermal and reflex agents affecting respiration. His scheme bridges the gaps and explains apparent controversial findings. Reflex control from exercised muscles is given a proper place in his theory.

Important investigations on respiration have come from the University Laboratory of Theoretical Gymnastics in Copenhagen. Asmussen *et al.* (5) experimented on a tabetic patient and found that although the lung ventilation was controlled by reflexes arising in working muscles, these reflexes evidently did not pass via the posterior fasciculi, since the latter are destroyed in this disease. The relative value of "humoral" and nerve control of respiration has also been investigated (6). Shutting off of blood circulation (by a pressure cuff) in working muscle causes no change in lung ventilation, although the oxygen consumption drops by 20 to 50 per cent. In voluntary hyperventilation at a constant alveolar carbon dioxide tension, about 3.7 to 5.4 per cent of the ventilation is used up to satisfy the increased metabolic needs (i.e., at a ventilation rate of 6 to 22 L. per min., 1.7 cc. of oxygen are utilized for each liter of ventilation; at the rate of 22 to 40 L. per min. the oxygen use increases to 3.0 cc. for each liter of ventilation) (7).

*Blood, circulation and content.*—During voluntary or electrically induced work, blood circulation increases in proportion to the amount of oxygen consumed, independently of the type of work. Thus, when a steady state is reached, the blood circulation is controlled by reflexes from working muscles rather than by impulses from the cortex (8).

Strenuous physical effort produces only minimal changes in the electrocardiogram of normal men (9). The tachycardia caused by muscular exercise does not change the ratio of the Q-T interval to the length of the cardiac cycle (10). Incidentally, a tachycardia as high as 320 beats per minute has been reported in well men during exercise (11).

It has been a common observation that training decreases the

pulse rate, and it has been considered an effect of training on the heart itself. Müller (12) gives evidence that this bradycardia is due to peripheral changes in muscles, i.e., better vascularization and oxygen utilization in the muscles used in training. The inference which may be drawn from this study is that if one wants to train for a given sport, for example track, it can hardly be done by practicing a different sport, for example swimming. The same investigator (13) suggests a formula for the estimation of the fatiguing effect of work, by means of a pulse quotient.  $PQ = \text{Pulse rate} / (25 \times \log O_2 \text{ intake})$ . The higher the PQ the greater is the fatiguing effect of work, the PQ limits being from 1 to 2.

Various attempts have been made to use the increase in pulse rate immediately after exercise for interpretation of circulatory conditions. This increase is often meaningless. Men in physically poor condition, with a high resting pulse rate, may have a smaller increase after strenuous exercise than men in better physical condition because their possible maximum pulse rate after exercise may be the same (14).

The epinephrine content of the blood in animals and men decreases during work, depending upon the degree of its intensity (15). This drop, however, is noticed only in untrained animals, the trained ones even showing an increase at times (16).

The blood sugar and serum magnesium decrease while running for two hours and quickly return to normal during recovery, after which the magnesium content drops again. The same changes are observed after injection of insulin, showing that changes in magnesium are probably bound with carbohydrate anabolism (17).

If exercise (bicycle riding) is stopped, blood congests in the dilated capillaries of the leg muscles (18). Mild exercise restores the "milking" effect of muscular contraction and aids in circulation. The value of mild exercise after violent exertion is known from experience—it is common practice to walk horses after a race, and athletes are also told to walk.

A drop in the cardiac output in the "vertical" position (60° on a tilting table) is not due to the mechanical disadvantages of such a position, but indicates that the presso-sensible reflex, which controls the blood pressure, does not control the heart output. If air, low in oxygen content, is breathed while the subject is in this position, the chemosensible reflex is evoked and the cardiac output is raised (19).

Men who have a tendency to faint in the standing position after a standard exercise may avoid fainting by breathing 4 to 7 per cent carbon dioxide. The explanation of the fact must be looked for in a higher blood pressure (20), because carbon dioxide in the vertical position does not increase the cardiac output (19).

The erythrocyte sedimentation rate five hours after strenuous exercise (five minutes of the Harvard Step-up Test) shows a statistically significant acceleration, but is back to the pre-exercise rate within twenty-four hours (21).

*Metabolism.*—The much debated question of the effect of training upon basal metabolic rate (B.M.R.) has been again investigated. In one report (22) it was stated that the B.M.R. declined after a period of training; in another report (23) that men who showed a good adjustment to physical exertion had a reduction in the B.M.R., while those who had difficulties in adjusting to training showed an increase in the B.M.R.

Most of the confusion regarding the effect of training upon the B.M.R. has been due to methodological errors. Very often measurements consist only of a single B.M.R. before and another after a period of training. Yet, it is well known that even two consecutive tests in one morning often show large differences.

The determination of energy cost of various exercises is one of the topics which is continuously receiving attention. In the reviewer's opinion these studies promise an important practical application in the complicated and more mechanized civilization of the future. Medical men should have an "exercise pharmacopoeia" for prescription of exercises to well and to sick people.

Erickson *et al.* (24) concluded, from treadmill studies, that a high degree of reliability is obtained from measurements of energy cost for speeds of 2.5 to 4.0 miles per hour and grades from 0 to 10 per cent. Variability in sixty-four pairs of tests was 2.95 per cent of the grand mean and was independent of grade and speed. The increase in energy expended between the combined extremes of speed and grade was 460 per cent.

Weiss (25) prepared a "cost list," based on oxygen consumption, of forty-two calisthenic exercises, using one subject. Although a subsequent study (26) done on thirty subjects showed considerable fluctuation in the cost, nevertheless the "cost list" may be used as a convenient guide for the prescription of exercises to convalescent patients. The unpredictable fluctuation in the various

physiological measurements (27) and in the cost of the same exercise for the same person needs further investigation.

Efficiency of work depends on oxygen supply and duration of the work. Anaerobic work is only 40 to 70 per cent as efficient as aerobic work and short spells of work are less efficient than long ones (28).

Walking for a champion walker is more economical than for an ordinary man. For him, running becomes less costly than walking only at 9.3 km. per hour; whereas, for the latter, the border line is lower (29).

*Warming-up and sweating.*—Of considerable interest are the reports on the value of warming-up that have come from Copenhagen. Warming-up before a contest is a common practice in athletics. The physiologic explanation of underlying reasons, however, has been somewhat vague, with an undue emphasis on the increase in blood circulation. Buchthal *et al.* (30) showed that muscle temperature depends on the magnitude of work and is due to aerobic heat production. Asmussen and Bøje (31) experimentally established that the rise in muscle temperature caused by a preliminary warming-up increases the work capacity of muscle and its tension during contraction. A passive increase in temperature by a bath, or by diathermy, also increases the capacity for work. The local rise in temperature is more important than the general increase in body temperature as measured in the rectum. Warming up the legs does not increase the work capacity of the arms, although the blood circulation in the arms is augmented. It takes about ten minutes of warming-up to reach the optimum. Incidentally, a preliminary massaging of the muscles does not increase their work capacity. This last observation should stimulate the advocates of massage in athletics to further studies in justification of preliminary massage.

The effect of external temperature upon work capacity of man has been an important topic of research during the war. Sweating plays an important role in heat dissipation, but when it becomes extreme, for example 1000 gm. per hour, the sweat glands may show signs of fatigue—the amount of sweating decreases and the body temperature may begin to rise (32). Heavy clothing reduces the efficiency of sweating and may cause heat exhaustion (33).

In this connection, one cannot help commenting upon the improvements in military uniforms during World War II. In some



areas, during hot weather, personnel were even allowed to remove neckties and to open collars. This has been criticized on grounds that such practice makes a soldier look "unmilitary." It is to be hoped that new uniforms for the Army and the Navy will take more into consideration the comfort of the men wearing them.

#### ERGOGENICS

By this term are meant agents of all kinds—physical, pharmacological, etc.—which cause an increase in work output. One may remark at this time that experiments with various dietary factors continue to be conflicting and not always convincing. It has been reported that the intake of grape sugar or grape sugar plus vitamin B<sub>1</sub> increases work capacity at normal external temperatures. However, if room temperature is as high as 39° C. only the sugar-vitamin combination causes an increase in endurance (34). On the other hand, a high carbohydrate meal about two hours before a short period of work (a 100-yard swim) produces no increase in the speed, as compared with that after a low carbohydrate meal (35).

The daily minimum amounts of each vitamin required for normal work output is still a debatable matter (36). The "indispensable" amount can evidently fluctuate greatly. A group of well-trained men showed no difference in work output and in the post exercise recovery rate of their pulse rate on two types of diet: one containing 0.85 mg. of vitamin B<sub>1</sub> and 0.95 mg. of riboflavin daily; the other, 3.06 mg. of B<sub>1</sub> and 3.47 mg. of riboflavin (37).

Observations made on Mohammedan children showed that when they skipped the noon day meal during the fasting period of Ramadan physical "skill" was decreased and endurance was not affected (38). Since the "skill" was measured by a 100-yard dash, the validity of this statement may be questioned. Naturally, prolonged semistarvation would cause a more profound deterioration in muscular fitness. In twelve to twenty-four weeks, the deterioration may be more than 70 per cent of normal fitness (39).

Injection of cortigen in untrained rats increased their work output (40). This seems to be logical since training causes a greater demand on the adrenal glands as evidenced by their hypertrophy.

The question of the effect of tobacco smoking upon athletic performance is a perennial one in athletics. Most coaches prohibit smoking during training, but the evidence for harmful effects is conflicting. The chief reason lies in the difficulty of estimating the

amount of smoke entering the organism. Moreover, some "experiments" have been based only on questionnaires. A recent report (41) states that, at certain levels of oxygen consumption during work, pulse rate is higher after smoking than without smoking. This may be considered as an indication of decreased oxygen carrying capacity of the blood, which, of course, would handicap a person during severe exertion.

A frequent argument against smoking is that smoking may shorten the span of life. This is evidently not applicable to rats, which live longer in an atmosphere of tobacco smoke (42). People ordinarily smoke for pleasure and for social reasons, yet often they have to forego this pleasure on account of serious pathological reactions. Since many millions smoke, extensive basic research on the effects of smoking on bodily health is warranted.

#### PHYSICAL FITNESS TESTS

Many definitions have been used for the term, physical fitness. From the standpoint of physical exercise, fitness means nothing more nor less than the degree of ability to perform a certain exercise. Naturally, the more types of tests given, the more exact the appraisal of over-all fitness. To economize on time, however, the number of items in a testing battery has to be reduced. This reduction leads to less thoroughness in appraising total fitness. Since different batteries include different items and advocates of certain tests become quite dogmatic about them and usually see the weaknesses rather than the good points in other tests, the short-cut tests provoke criticism. The curious thing is that fairly similar results may be obtained using different sets of such tests, provided they are used consistently and correctly.

During this war, two tests were especially widely used: (a) the Army Air Forces (AAF) Test (43 to 48) and (b) the Harvard (Brouha) Step-up Test and its modifications (27, 49 to 56, 82). However other tests are also in use. [The AAF test consists of sit-ups (up to 114), chinning (as many as possible), and a 300-yard shuttle run (43). This test can easily be administered to large groups. The reliability is 0.82 (43).] Convenient graphs have been prepared showing the relation between the scores obtained in this test and age (44). The main drawback of this test has been the requirement of an outdoor 300-yard run, which has made it unusable during inclement weather. Subsequently, an indoor run was

introduced. There seems to be general agreement that a battery of test items should include at least one type of strenuous exercise, in terms of horsepower output. Although running has remained the most widely used item, stepping-up has proved convenient on account of its simplicity and adaptability to indoor and outdoor conditions. The correlation between the Harvard Step-up Test and running, however, is not very high, being only  $+0.38$  (50).

A surprisingly low coefficient of correlation ( $+0.082$ ) has been obtained, also, between the stepping-up test and the amount of work done in two minutes on a bicycle ergometer (56). One must remember, however, that the weakness of the bicycle ergometer as an instrument for measuring work capacity lies in the fact that the subject must practice for some time beforehand; otherwise, the results obtained may be questioned.

A great deal of work in establishing intercorrelations between the various testing items has been done by Cureton and his co-workers (57, 58). The knowledge of intercorrelations helps in constructing new batteries of tests, and determines the feasibility of substituting one test for another. For example, leg-lift and sit-up tests have a great kinesiologic similarity, yet the coefficient of correlation between these two tests is lower than one might expect, being  $+0.38$  (59), showing that the two are not interchangeable in a battery of tests.

Interest in breath holding as a test of physical fitness has increased recently. The author, nevertheless, agrees with the old statement of Hambley *et al.* (60) that breath holding only measures ability to withstand the discomfort caused by this test. Breath holding cannot be used for predicting endurance either in running or in the Harvard Step-up Test, because the coefficients of correlation between breath holding and these two tests are not statistically significant (53). Elbel (54) studied the Flack breath holding test (blowing against a 40 mm. column of mercury) and found that it correlates only  $+0.237$  with running to exhaustion on a treadmill. Since breath holding depends so much on will power, it has been used in diagnosing anxiety states (61). If a subject is required to hold his breath "until a moderate degree of discomfort is experienced," as has been suggested (62), the end point of the test becomes indefinite, and the results may be questioned.

Speed of the knee jerk has been suggested to measure the degree of deterioration in physical fitness. It has been shown that starvation and deficiency in thiamine slow down the kick. It is in-

teresting to observe that no correlation exists between the strength of the muscles and the speed of contraction (63).

Several other tests may be briefly mentioned. Postural steadiness as measured by the ataxiometer gives more information regarding fatigue after a run than pulse rate (64) and so serves as a measure of physical fitness. A test consisting of holding arms outstretched sidewise and subsequent hopping, and their effects on pulse rate, has been proposed for diagnosis of psychoneurotic patients (65). This test is claimed to be as good as a thirty-minute conventional examination. The change in electric conductivity of the skin of the palms, proposed recently (66) as a test of physical fitness, was thoroughly investigated by Elbel & Ronkin (67) and found wanting.

During the war the author found no evidence to indicate that outstanding athletes are more successful as flyers. He proved only that there is a definite relation between man's coordinating ability in exercising and success in learning to fly (68), which was subsequently substantiated by Turk (69). Graybiel and West (70) showed that among the naval flight students differences in physical fitness had no effect upon success in flying training.

It would be desirable to have a physical fitness test sensitive to mild changes in health and nutritional status. Such a test has yet to be found. In a test consisting of a shot put, and 100- and 600-yard runs used on children in South Africa (72), even an "extremely bad" state of health was not reflected in the results (73). The authors draw an obvious conclusion that "physical fitness" is not necessarily destroyed even by severe illness, but the conclusion that the test is too crude might also be drawn. It is conceivable that a test involving great physical effort would detect deterioration in fitness when one demanding slight physical exertion fails. Yet as strenuous tests as the shot put and the runs in the above example may fail to reveal an expected and acknowledged decline in fitness even during disease. It is clear that we must continue to strive to find a test which can better reflect a state of health.

Reports evaluating physical training programs "in the field" should be accepted with extreme caution because of the almost unsurmountable difficulties in gathering adequate data. Studies have shown that the differences in physical training programs in the AAF as to content and number of days participation per week were overshadowed by the "human equation," which depends upon personality of instructors and interest of participants (47, 48).

*Race and sex.*—Studying the records of the 1936 Olympic games, Jokl (74) comes to the conclusion that Negroes are more adapted to athletics and thus are more physically fit than white people. He calls attention to the fact that the United States received eight times more points than it would have if the points had been distributed merely in proportion to the size of the populations in the participating countries and that Negroes from the United States received fifty times as many points as they were entitled to on the same basis. Studies conducted in South Africa showed that Bantu boys and girls score higher on physical fitness tests than white boys and girls of the same locality in spite of the fact that Bantus live on a lower economic level than the whites and their dietary conditions are often inadequate (75).

Women are often superior to men in skills but inferior in strength and endurance (76). Girls, in South Africa, who start menstruating early are "bulkier" than nonmenstruating ones. They also seem to be better at the shot put and worse at the 600-yard run (79). The aerobic work capacity of women in walking is 61.5 per cent that of men (78). Swimming for women develops endurance better than other sports, as judged by the Burpee test (79).

Physical education programs in the schools are still based upon a combination of facts and fancies, and fancies appear to predominate in determining the program for women. Ramsey (80) blames the retroverted uterus and cystic degeneration of ovarian tissue on physical exercises. She would eliminate from the physical education program for girls "sudden jumping from still position or any type of vault exercises." Studies like this one indicate the definite need for research in medical aspects of physical education.

*Exercise at altitude.*—Muscular exercise at 38,000 feet of simulated altitude hastens and increases the incidence of "bends," which appear mostly in the exercised limbs. Time of onset and severity are related to the amount of work done (81). At high altitude, men can readily develop mild states of anoxia; heavy muscular work may cause a greater depletion of oxygen in the blood for this reason (83), thus raising the oxygen debt and retarding the period of recovery (84).

*Exercise in the rehabilitation of convalescents.*—Military regulations have required, and rightly so, that men could not be discharged from a hospital until they were able to perform their duties. However some medical men questioned the wisdom of

indiscriminate bed rest, and clinical experience indicated possible dangers arising from the abuse of bed rest. The studies pertaining to this topic were well summarized in a series of reports (85 to 90). On the initiative of Rusk (91) a new program of physical reconditioning for convalescents was started in the AAF hospitals and eventually spread through the other Army and Navy hospitals. Many such programs have been proposed, but only a few are mentioned here (92, 93, 94, 95). Most of these programs have been based on "personal experience" because of the difficulties involved in experimentation.

Of special interest is work in reconditioning adult patients convalescing from rheumatic fever (96, 97). The frequent incidence of cardiac damage, so common to this disease, has resulted traditionally in prolonged bed rest and prohibition of physical activities. A program of graded, well-controlled physical activities tried on adults recovering from rheumatic fever has resulted in a marked drop in psychoneurotic manifestations and a rapid improvement in physical fitness without an increase in the incidence of cardiac lesions. The testing records have been very useful for the determination of the fitness of patients for duty at the time of discharge from the hospital (97).

#### COMMENTS

The goal of research in the physiology of exercise is to determine:

1. The effect of physical activities not only upon the functioning of various physiological systems, but on man's total health and well being.
2. The kind and the amount of exercise needed by an individual in accordance with sex, age, and body type under various life conditions.

At present, one can count on the fingers of one hand all the laboratories in this country where these problems constitute the major part of research. It seems obvious that more laboratories should be established. Moreover, there is need for an Experimental Institute in Health and Physical Education.

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SPRINGFIELD COLLEGE  
SPRINGFIELD, MASSACHUSETTS

## THE VISCERAL FUNCTIONS OF THE NERVOUS SYSTEM\*

BY W. R. INGRAM

*Department of Anatomy, The State University of Iowa, Iowa City, Iowa*

### INTRODUCTION

It seems apparent that there must be exceptions to any concept of the autonomic or visceral portion of the nervous system. The weaknesses of the old concept of balance between two antagonistic divisions are well established, and much can be said for more liberal viewpoints. However, the theory of dual innervation has not as yet been rejected by the majority of clinicians and physiologists and it must be admitted that all the facts necessary for its complete abandonment are not as yet forthcoming. Workers in the field are aware of its pitfalls and feel free to make necessary adaptations.

The study of the visceral nervous system in man seems to be advancing. Not only are the clinicians applying the findings of the laboratory, but they are making, in many instances, carefully controlled observations whereby knowledge of the physiology of the human nervous system may be secured upon a firmer basis. This observation is one of several rewards of a survey of the recent literature.

### ANATOMY

There has been much emphasis recently upon study of the anatomic organization of the autonomic system in man. This emphasis is understandable in view of the number and variety of surgical operations now performed on the vegetative system. The latter fact has moreover given the resourceful observer an opportunity to ascertain anatomic facts not readily susceptible to conventional attack. Thus, Richter and co-workers (1, 2) have studied sympathetic dermatomes by means of the electrical skin resistance method in patients who had submitted to thoracolumbar or lumbosacral sympathectomies. The patterns of increased resistance on the lower extremities were much more constant than

\* This survey covers the period from August 1945 to July 1946, with the inclusion of some earlier reports which have not hitherto been available.

in the thoracic and abdominal regions, where there was considerable variation and marked overlap of the segments, with occasional strange gaps where normal innervation persisted. In general the patterns conformed to the sensory dermatomes as outlined by Foerster. The variability of peripheral distribution is more than matched by the configuration and arrangement of sympathetic ganglia and rami (3, 4). The frequency of fusion of ganglia, and the variation in number of communicating rami are striking (4). These variations are especially marked in cervical and lumbar regions. It is evident that the textbook descriptions are too conventional.

A carefully illustrated study of the pelvic autonomic nerves in the male (5) indicates that these nerves are so disposed as to be ordinarily protected from damage in urologic surgery. The distribution of extrinsic and intrinsic nerves to the uterus has been restudied (6); no ganglion cell bodies were found in the uterine wall.

On the microscopic side, it is of interest that approximately two thirds of the fibers of the chorda tympani of the cat and dog are sensory (7) and relatively few are unmyelinated. Blood vessels of the bone marrow receive numerous sympathetic and afferent nerve fibers (8), which traverse the nutrient foramina. While afferent fibers and endings are found in the parenchyma, there is no evidence of innervation of parenchymatous tissue by efferent fibers. These findings are supported by Foa (9), who observed that stimulation of nerves to marrow caused it to be reduced in volume, with a "squeezing" of mature and immature blood elements into the blood stream. The volume change was ascribed to vasoconstriction. Denervation of limbs in rats did not prevent epiphyseal growth of bone (10).

The acinar and islet cells of the pancreas are innervated only through parasympathetic fibers, while the sympathetic components of the pancreatic nerves all pass to blood vessels (11), as is probably true for most glands. Myelinated fibers following intrarenal vessels have been seen in human kidneys (12).

Ganglionic neurons containing what appeared to be secretion granules were found in small periadrenal ganglia in the monkey (13), but not in other peripheral ganglia.

The view that separate cellular intermediaries are necessary for formation and release of the substances involved in chemical

transmission is sometimes expressed. A study of glandular innervation by Champy *et al.* (14, 15, 16) supports this view. It is claimed (17) that the osmium iodide staining method of Champy is specific for epinephrine. This is said to make possible the differentiation of adrenergic structures from neural and connective tissue elements. A terminal intercellular network formed of processes of certain black-stained interstitial cells is described. This network, it is suggested, releases epinephrine when stimulated by nerve impulses reaching it through sympathetic fibers—thus it is unnecessary to assume a separate innervation for each gland cell. These interstitial cells are said to be incompletely differentiated neurons, as originally described by Cajal. More specifically, the findings indicated that the innervation of the salivary glands and pancreas is mainly adrenergic. The method was not successful with sweat glands because of the cholinergic innervation of the latter. While this work appears to have been carefully done it needs to be carefully checked because of some apparent inconsistencies in results. The method is admittedly capricious. The conclusions as to the adrenergic innervation of salivary glands and pancreas are at odds with most physiological and anatomic findings (11). Adrenergic synaptic relationships are implied. No experimental approach has been used; the findings after selective extrinsic denervation would be interesting. Denber (18, 19) did not find a terminal reticulum in the adrenal, but observed some peculiar large club-shaped terminals which he believed to be associated with Lavrentiev's cells.

The connection between mammillary bodies and the gyrus cinguli and medial surface of the frontal lobe has been demonstrated electrically in cat and monkey (20). The strychninization method has also yielded information as to hypothalamocortical relationships (188). In three cases, degeneration of the small celled portion of the dorsomedial nucleus of the thalamus has been found following prefrontal lobotomy; the large celled portion which presumably connects with the hypothalamus remained intact (21). It is suggested that the effect of the operation on emotionality is due to interruption of this connection. In nineteen cases with carcinoma, cellular degeneration was found in certain conspicuous hypothalamic nuclei (22).

Experiments involving extirpation of neural crest in the chick have been extended to the thoracic and lumbosacral regions and

the local origin of sympathetic ganglion cells from neural crest is reaffirmed (23). The thoracolumbar autonomic system of the fetal whale has been described (24) and it was found that the inter-mediolateral cell column of the spinal cord does not descend below T1. The innervation of insect hearts has been studied (25).

#### VISCERAL AFFERENTS

It is generally taught that visceral afferent neurons resemble the somatic in having their cell bodies in spinal ganglia. Their distal axons may traverse sympathetic nerves. Successful attempts to relieve pain of visceral origin by sectioning appropriate sympathetic nerves are frequently made. Two possibilities exist: (a) pain is relieved by sectioning fibers bearing pain impulses; (b) pain is relieved by sectioning efferent fibers, the abnormal activity of which sets up a peripheral situation whereby afferent fibers of other nerves are stimulated. An example of the first is section of the hypogastric plexus or the lumbar chains (26 to 30) for relief of uterine pain; of the second, relief of pain of gastric ulcer following bilateral vagotomy (99). Certainly such effects of efferent denervation should not be ignored.

Relief from phantom pain after amputation may follow sympathectomy or sympathetic block in some cases (31), although many of these cases are undoubtedly cerebral in origin.

A stimulating suggestion as to a cause of posttraumatic and causalgic pain has been offered by Doupe and co-workers (32), who believe that impulses in efferent sympathetic fibers may stimulate adjacent sensory fibers in the same nerve. This implies that the sensory fibers must have been altered in some way by the injury or pathological process so as to become accessible to such stimulation. Antidromic impulses in the sensory fibers thus stimulated could be responsible for the vasodilatation which often accompanies posttraumatic pain. Experimental support for this concept may be derived from the setting up of "artificial synapses" at the damaged area of a severed or compressed nerve as shown by Granit *et al.* (33). A similar interaction may account for the pain produced by tactile stimulation of a skin area the nerve supply of which has been previously compressed. The pain fibers in the compressed region would be stimulated secondarily by the action-potentials of the nearby tactile fibers (34). Injury potentials in splanchnic afferent fibers have been shown to be increased by

overventilation and inhibited by rise in carbon dioxide tension of the blood (34). Similarly, the pricking paresthesias following restoration of circulation to a compressed extremity are reduced by hypercapnia.

An excellent discussion of pain following peripheral nerve injury is given by White (35). Certain aspects of pain are also considered in the chapter on cutaneous senses in this volume.

#### VISCERAL EFFERENTS

*Heart.*—While it is generally acknowledged that the heart is richly supplied with extrinsic and intrinsic innervation and that there are extensive atrioventricular nervous connections, the contention that Purkinje fibers and myogenic conduction are not significant in human hearts (36) has not as yet been accepted. The existence of Purkinje tissue and the atrioventricular bundle in the human heart has been affirmed (37, 38) as has the continuity of Purkinje and cardiac muscle fibers (38). It should perhaps be noted, however, that the investigators did not find such tissue in all cases, and it seems evident that the left bundle branch is weak and variable and difficult to find. The atrioventricular node was dissectible in only five of twenty hearts (37). Some aspects of the work of Lavrentiev (39) have recently been reviewed. It is interesting to note that while many investigators have found strong sympathetic distribution to the ventricle, Lavrentiev thought sympathetic fibers were restricted to the atria. Another point of interest is his description of myelinated fibers which bifurcate, one branch going to a sensory ending on a coronary vessel, the other to one or more muscle fibers of the myocardium, setting up an axon reflex arrangement. The sensory innervation of coronary vessels is very rich.

Vagal stimulation of the intact turtle heart may cause local fibrillation and also tends to block sinoauricular transmission (40, 41). Camphor prevents vagus action on the turtle heart (42), presumably by desensitizing the muscle fibers to acetylcholine. The only significant direct action of atropine on cardiac muscle of the turtle seems to be depression of conductivity at high rates of beating (43). The immediate source of acetylcholine in rabbits' hearts is a labile precursor (44), which is transformed upon vagal stimulation. While prolonged stimulation reduces the content of precursor, vagal escape, which is confined to the ventricles, is not

due to lack of precursor, nor to inhibition of transformation of the latter.

Acceleration of the heart resulting from administration of acetylcholine in atropinized dogs with sympathectomized hearts is not due to fall in blood pressure, but is very likely due to reflex stimulation of vagus cardioaccelerator fibers or direct intracardiac stimulation (45, 46). Contrary to other reports, Cicardo & Gurevich (47) found no increase in potassium content of blood plasma drawn from the left ventricle of the intact heart during vagal inhibition or ventricular fibrillation, nor did the blood phosphorus change under these conditions. Traumatic shock in dogs was said to be ameliorated by intracisternal injection of potassium phosphate (48). This result is supposedly due to stimulation of bulbar vasomotor and cardiac centers. A more detailed study of intracisternal injection of this substance (49) showed that small doses may decrease blood pressure and heart rate, while large doses have an opposite effect; sinus arrhythmias also occurred in intact animals, a variety of abnormalities in vagotomized dogs. Incidentally, the expiratory and inspiratory portions of the respiratory center were activated in succession, perhaps because of differences in their positions, and apneusis could be produced in dogs after vagotomy or pontile transection. In general the effects of potassium phosphate were stimulatory, reversals in effect being due to activation of opposing centers. Cardiac sympathectomy abolishes spontaneous ventricular arrhythmias due to cyclopropane (50), since normal sympathetic tone is necessary for the occurrence of these irregularities.

The effect of lumbodorsal sympathectomy on the electrocardiogram in cases of hypertension has been carefully studied in a large series of carefully controlled observations by White and co-workers (51, 52). A conservative evaluation of cases in which diastolic blood pressure was lowered by this procedure indicated very strongly that the secondary cardiac changes as manifested by the electrocardiogram are often greatly improved, perhaps due to lightening of the load upon the heart, although more direct physiological reasons for the improvement remain to be discovered. Similar results were reported by Peet & Isberg (65).

Fauteux (53) found that survival rate after coronary ligation was greatly increased in dogs which had previously been prepared by pericoronary neurectomy and coronary venous ligation (the



latter to promote collateral circulation). The application of this procedure to a patient suffering from coronary disease with angina was followed by improvement and cessation of pain.

*Blood vessels.*—It is implied that the sympathetic control of venous tone is of considerable importance in regulating cardiac output and hence blood pressure (54). The fall in arterial pressure caused by stimulation of the carotid sinus nerve or spinal cord section is said to result from decreased cardiac output. The latter is due to diminished venous return consequent to dilatation of the capillaries and veins. Use of the digital plethysmograph (55) indicates that generalized vasodilatation occurs during anesthesia. Regional dilatation in nerve block or spinal anesthesia is normally compensated for by vasoconstriction elsewhere. In hemorrhagic and traumatic shock, peripheral circulation declines before the blood pressure falls; hence the method affords a valuable indication of impending shock. Changes in volume of the hand and foot in simultaneous plethysmographic recordings were remarkably parallel (56), indicating the relative importance of central nervous control as against peripheral and local factors in the regulation of peripheral circulation. When the temperature of the hand is controlled, its blood flow is related to the temperature of the body (57), especially in moderately cold hands ( $15^{\circ}\text{C.}$ ), the flow being greater the warmer the body.

Page (58) has summarized the results of experiments which indicate that a rather generalized vasoconstriction accompanies terminal shock. The vasoconstriction is initiated by vasomotor reflexes but maintained by the action of an ultrafiltrable substance which is formed at the site of tissue injury and which appears in the plasma. Vasodilatation does not appear until shortly before death.

The sudden fall of blood pressure in post-hemorrhagic fainting is due to vasodilatation in skeletal muscles, according to Barcroft *et al.* (59, 60). In significant experiments these investigators induced fainting in human subjects by application of venous tourniquets to the thighs, followed by bleeding as much as 840 cc. Blood flow in the hand and in the forearm was recorded by a plethysmographic method. Cardiac output was estimated by a direct Fick method. Blood flow in the muscular forearm increased as the blood pressure fell, in contrast to that in the relatively unmuscular hand. Since there was no significant change in cardiac



output, the vasodilatation thus probably occurring in all the skeletal muscles was sufficient to account for the fall of blood pressure in hemorrhagic fainting. Blocking of the nerves to the forearm greatly diminished this vasodilatation, indicating that it is an effect of activity of the vasomotor center by way of vasodilator fibers. Failure of vasodilatation in sympathectomized forearms showed that these fibers traverse the sympathetic ganglia and that the phenomenon is not due to vasodilator impulses traversing dorsal roots, nor to epinephrine. Muscle tone was not a factor because muscular paralysis induced by nerve block in sympathectomized subjects did not increase blood flow in the forearm. This is the first evidence indicating existence of sympathetic vasodilator nerves to the vessels of skeletal muscle in man. The sources of error in measurement of cardiac output by the method of right atrial catheterization, as discussed by Warren *et al.* (61), should perhaps be considered in evaluating these experiments.

Direct stimulation of the renal artery and its accompanying nerves has been carried out in dogs (62). Short periods of stimulation reduced renal blood flow seventy-five per cent but did not raise arterial pressure. Stimulation for twenty-two hours a day produced a rise in blood pressure which was maintained only while daily stimulation continued, or for as long as twenty-seven days. The cause of the hypertension has not as yet been determined, but it is not due simply to hemodynamic changes in the kidney. Ogden and co-workers (63) have summarized the evidence which leads them to suggest that while experimental renal hypertension is humoral in origin, humoral factors do not participate in its maintenance over long periods. Persistence of chronic hypertension depends upon neural mechanisms, because factors lowering blood pressure in early hypertension are not effective in hypertension of long duration, while persistent hypertension in rats is relieved by procedures affecting the sympathetic system, such as treatment with pentobarbital, yohimbine and F 883. While conclusive direct evidence is not as yet forthcoming, the idea is suggestive and perhaps applies to those cases of hypertension in man not directly related to nephropathy and which are relieved by sympathectomy.

The results of large numbers of lumbodorsal sympathectomies performed for relief of hypertension are now being evaluated by the surgeons (64 to 68) and the details of these analyses need not be discussed here. In general it may be said that favorable results

have been obtained in cases in which irreversible vascular changes have not become too widespread. The real reason for the lowering of blood pressure is not yet entirely evident. Apparently changes in peripheral blood flow are not significant (66). A splendid discussion of sympathectomy as related to the circulation, stressing fundamental anatomic and physiologic considerations has been published by Grimson (69).

Physiological methods in selection of hypertensive patients for sympathectomy have been applied (70, 71). Exercise produces a fall in diastolic pressure after thoracolumbar sympathectomy in man (72). Gorev & Smirnova-Zamkova (73) found a change in the physical state of argyrophilic substance in the walls of blood vessels in experimental neurogenic hypertension. This may impair the filtering capacity of the vascular wall. With persistence of the hypertension, the argyrophil substance is transformed into collagen and fibrosis results. Even in cases of peripheral arteriosclerosis, lumbar sympathectomy may improve circulation in the extremities (74, 75). The procedure is also of value in thrombophlebitis and chronic ulcerations (76, 77), as well as after obstruction of major arteries in the lower extremity (78). In dealing with peripheral nerve injuries (postganglionic) it is necessary to recall that autonomic effector organs may in time recover a degree of reactivity to local conditions (79). Thus the caliber of blood vessels becomes dependent upon their environment, and the denervated skin may become cold in certain climates. Axon reflexes may be present after preganglionic injury. Vasodilatation accompanying causalgic pain may in part be due to antidromic vasodilator impulses set up by cross-stimulation of fibers in injured nerve (32). Regional sympathetic block may relieve the pain of herpes zoster, presumably by interrupting a vicious cycle of impulses originating in the infected spinal ganglion and resulting in segmental vasospasm (80). Curare is capable of producing hypotension (91).

The functional innervation of veins is essentially the same as that of arteries (81). Pain of venous origin as well as venospasm may be relieved by local vascular anesthesia or sympathetic block. Sympathectomy or repeated sympathetic block may promote an increase of collateral circulation in thrombophlebitis.

Pulmonary edema may occur as a result of disease of the central nervous system, especially of the vagus region. In a case of penetrating wound of the medulla oblongata, the terminal edema was

held to be due to increase in capillary permeability because of loss of vagal influence as well as to partial interruption of respiratory pathways (82). Wassermann & Goodman (83) reject the classic theories of the genesis of pulmonary edema and relate it to autonomic imbalance, especially to disturbance of the sinoaortic apparatus. They say that acute attacks of pulmonary edema can be checked by pressure upon the carotid sinus. On the other hand Luisada & Sarnoff (84, 85) found that sympathectomy or denervation of the carotid sinus tended to increase resistance to experimental pulmonary edema. They concluded that the edema may be due to the setting up of a carotid-pulmonary reflex mediated by the ninth nerve and the sympathetic system which leads to increased permeability of pulmonary vessels.

*Digestive system.*—The extrinsic innervation of the muscle fibers of the lower part of the esophagus is entirely from the vagus (86, 87), fibers which also enter the intramural plexus. The latter can function after vagotomy, however. The sympathetic supply is limited to blood vessels. A connection between the vagus and the left suprarenal plexus contains sympathetic fibers arising from the latter and passing to blood vessels of lower esophagus and stomach (88). Experimental and clinical evidence has been advanced to show that stimulation of the right splanchnic causes loss of tone and motility of the gall bladder and biliary tract, while section of the same nerves permits improved evacuation. Vagus section caused dilatation (89). Right splanchnicotomy in twenty-three cases is said to have increased motility of the gall bladder and improved digestion and subjective sensation. While somewhat optimistic, this work does not conflict greatly with that of Boyden, reviewed by Freeman (155).

Ephedrine inhibits the effects of acetylcholine, but not of histamine, on rabbit's intestine, apparently by acting on post-ganglionic terminals (90), especially when intestinal tone is maximum. It is said not to interfere with normal peristalsis, ordinarily, as atropine does. Curare causes relaxation of the muscle of the small intestine (91), due in part to direct effect on the effector mechanism. It seems evident that curare may act anywhere in the neuromuscular system where acetylcholine is the chemical mediator. There is evidence that ileus may be caused by reflex inhibition or spasm in response to painful stimuli elsewhere

in the body cavity as well as to local stimuli (92). Caffeine produces an increased output of acid by the cat's stomach after bilateral vagotomy or 1 mg. of atropine sulphate (93). The action is apparently peripheral, but a central action may also be present. It is of interest that the dog's stomach does not respond to caffeine. Motility of the colon increased after either small or large hemorrhage in dogs, but that of the stomach and small intestine was either not affected or was depressed (94); fall in blood pressure was not believed to be a factor.

Anesthetization of sympathetic nerves often relieves spastic colitis, perhaps because of interruption of afferent elements concerned in production of reflex spasm (95). The effects of localized infiltration of the sympathetic system in cases of megacolon indicate that the cecum and ascending colon are innervated from the twelfth thoracic and first lumbar ganglia, the transverse colon and splenic flexure from the first three lumbar ganglia, and the descending colon and sigmoid from the lower lumbar. Relief of dilatation of the descending colon necessitates bilateral resection (96, 97). These findings are probably indicative, but the anatomical variability of the sympathetic chains must be recalled (4). While the splanchnics are not ordinarily held to be directly concerned in colon innervation, splanchnectomy was of benefit in three out of five cases of megacolon congenitum (98).

Supradiaphragmatic section of the vagi has now been done in enough cases of peptic ulcer that the surgeons feel justified in considering it a promising and highly beneficial therapeutic procedure (99, 100, 101). It is physiologically significant that while this operation greatly reduces gastric motility, appetite and hunger sensations remain normal. Furthermore, while pre-existent pain is eliminated, pain sensation itself is not lost (101), and the pain pathway must follow sympathetic paths. While acid secretion by the fasting stomach is greatly reduced, the values for combined acid remain relatively high, perhaps indicating that neutralization of acid is facilitated (100). Digestion of food is more complete because of the prolonged emptying time. Vagotomy has practically no influence upon the secretory response to histamine and caffeine (99), but abolishes the response to insulin hypoglycemia. The central nervous reflex mechanisms affected by insulin hypoglycemia have not as yet been located or determined. A contrasting

approach to the treatment of gastroduodenal ulcers has been reported by Froehlich (102) who performed splanchnectomy and removal of the first lumbar ganglion with reported success in fourteen out of twenty-five cases. Little laboratory data is given and it is possible that simply the pain pathway was affected. The well-known psychosomatic elements in gastric ulcer must also be recalled. That vagotomy may cause gastric ulceration in rabbits has been reaffirmed (103), and it also appears that vagotomy does not protect against gastric ulceration produced by chronic histamine stimulation in dogs and cats.

Von Pongracz (104) reports that denervation of the pancreas promotes signs of degeneration of islet cells and of the acini, although he observed only a slight hyperglycemia. He does not believe, however, that insulin secretion is under nervous control through the vagus nerve, contrary to the findings of Gellhorn and others. After prolonged stimulation of the vagus or administration of pancreozymin, enzyme granules in pancreatic cells decreased in number but complete exhaustion was never attained (105). Inflammatory lesions of nervous tissue in the region of the pancreas have been found in chronic pancreatitis (106). Edematous pancreatitis, with intense congestion, hemorrhage, and cellular degeneration, has been produced in guinea pigs by left splanchnic nerve stimulation (107). Section of the left splanchnics in cases of chronic pancreatitis has been reported to afford remarkable relief (108, 109).

*Urinary organs.*—Bilateral lumbar sympathectomy has been reported to yield favorable results in a case of postoperative megaureter (110). The case of a male patient on whom bilateral resection of the superior and inferior hypogastric plexuses was performed for relief of cystalgia is of interest in that sexual function was apparently unaffected (111). Renal colic has been relieved by lumbar sympathetic block (112).

Kuntz & Saccomanno (113) have used a technique of Nonidez in restudying the innervation of the detrusor musculature of the bladder. This technique is said to permit distinction between pre- and postganglionic fibers because of difference in staining intensity. These workers found postganglionic, and hence sympathetic, fibers in the bladder wall in larger numbers than would be required for innervation of blood vessels, and while they did not actually

see terminals in muscle fibers, they concluded that the detrusor musculature receives sympathetic as well as parasympathetic innervation. They did not consider the possibility that some of these fibers might originate in intrinsic ganglia. Stimulation of the sympathetic supply of the bladder in dogs, prepared in such a way that the basal musculature could not affect the records of contraction, produced an initial light contraction followed by prolonged inhibition. These findings are at odds with the earlier work of Langworthy *et al.* (114), who believed that the sympathetic innervation of the bladder is concerned only with the trigone region. A question may arise as to the validity of the staining method as applied here.

In a case of Erb's spinal paralysis with symptoms confined to the bladder, McMichael (115) found degeneration in the dorsal and superficial portions of the lateral funiculi, indicating the position of the central bladder pathways in the human spinal cord.

*Blood.*—Vague changes in blood picture after sympathectomy have been reported (116). Nervous effects on blood clotting are probably not concerned with change in plasma prothrombin because administration of epinephrine in dogs and rats produced no significant change in prothrombin time, either in normals or in dogs rendered hypoprothrombinemic by dicumarol (117).

*Endocrine glands.*—A study of the innervation of the hypophysis in the armadillo (118) has confirmed the contention of those who believe that few fibers enter the anterior lobe from the infundibular stem or process, while a rather abundant sympathetic innervation was found. Terminals of the latter establish contact with glandular cells. Brolin (119) has reported at length on studies of pituitary and thyroid changes in rats exposed to cold. He has confirmed the older observation of Uotila that thyroid activation in response to cold is not affected by cervical sympathectomy but is abolished by section of the hypophysial stalk. The activation of the thyroid depends upon a basophilic reaction and increased output of thyrotropic hormone by the anterior lobe. This is lost in stalk-sectioned rats and thyrotropic function is reduced also in such rats even when not exposed to cold. Prolonged exposure to cold induces changes in basophilic anterior lobe cells similar to those ensuing after thyroidectomy. It is possible that adreno-corticotrophic hormone production may also increase under cold

stimulation. A reason for these effects has not been offered as yet. An observation at odds with the findings of other observers was that stalk section appeared to cause a reduction in gonadotropic hormone production, especially in male rats. A matter of the character of the operation is probably involved here.

*Dorsal root efferents.*—It has long been established that peripheral vasodilatation follows stimulation of dorsal roots and it has been held that this is due to antidromic impulses. Bach (120, 121, 122), however, has presented evidence to indicate that dorsal root participation in reflex vasodilatation following stimulation of the depressor nerve is carried out over efferent fibers which form synapses with postganglionic neurons in the dorsal root ganglia. These parasympathetic-type fibers are said to maintain a degree of vasodilator tone and to function cholinergically. A depressor reflex could not be produced following avulsion of all the dorsal roots from the cord in cats. The evidence for existence of synapses in dorsal root ganglia was derived from the finding that vasodilatation could not be produced by stimulation of the peripheral cut end of the dorsal root after injection of nicotine into the corresponding spinal ganglion in frogs. The difference from earlier results of Bayliss and others was ascribed to the injection technique. These results are of great significance if the procedures prove to have been properly controlled. Anatomical evidence for synapses in dorsal root ganglia is not good, and certain authorities strongly deny that efferent dorsal root fibers exist.

*Humoral mechanisms.*—Acetylcholine is present in resting muscle and in hearts as a labile "acetylcholine precursor" (123, 124). Vagal activity releases acetylcholine from this complex, but even without vagus action there is constant breakdown and formation of precursor. Part of the freed acetylcholine is resynthesized into precursor. A parasympathomimetic substance resembling acetylcholine has been found in normal human aqueous humor (125); it is absent in cases of glaucoma. An antigenic action on terminal organs releasing acetylcholine is said to play a role in anaphylactic shock (126). Loewi (127, 128) has recently discussed certain aspects of chemical transmission, emphasizing the necessity of this concept for understanding peripheral inhibition. Burn (129) has reviewed the evidence for epinephrine potentiation of acetylcholine transmission in ganglia; large doses of epinephrine, however, suddenly released, depress ganglionic transmission.



After-discharge from sympathetic ganglion cells appears due to persisting environmental change or to changes in the cell body induced by activity of presynaptic endings (130).

### REFLEXES

The concept of reverberating reflex circuits as applied to reflex sympathetic dystrophy has been clearly introduced into clinical thinking by Evans (131). The vasomotor systems of four of ten subjects have been conditioned to light stimuli (132). A central vagal reflex center is localized in the expiratory portion of the respiratory center (133). Selective elimination of aortic and carotid chemoreceptors reduces ventilation thirty-five per cent in cats, and abolishes any effect of carotid compression on respiration (134). Efferent discharges over the splanchnics are greatly influenced by activity or suppression of the buffer nerves and by changes in the body fluids, according to Gernandt and co-workers. For instance, section of sinus and vagodepressor nerves increased electrical activity in the splanchnic (135). Recordings of action potentials from the splanchnic nerve indicates that ergotamine abolishes the inhibitory influence of higher areas upon spinal vasomotor centers (136). Hyperactivity of the cardio-inhibitory carotid sinus reflex is not due to afferent hypersensitivity but to increased reactivity of the center or efferent path (137). Bilateral cervical vagotomy does not eliminate the electrocardiographic change produced by pulmonary emboli (138), hence there is no evidence for existence of a vagal pulmonocoronary reflex. After prolonged anoxia has induced a state of vasodilatation, administration of excess oxygen causes a further fall in blood pressure (139). This reaction is due to an effect on bulbar reflex mechanisms; perhaps because of release of overactive chemoreceptors. It is abolished by section of the vagus and carotid sinus nerves. Stimulation of vagus afferents in the rabbit most frequently depresses blood pressure and respiration, but does not affect heart rate (140). Compression of the epigastric region causes a fall in pulse pressure, according to Grandpierre and co-workers (141). Experiments of Grimson (142) show that vasomotor control is largely abolished in completely sympathectomized dogs, but that after several years an incomplete recovery occurs. Chemoreceptors affecting blood pressure have been demonstrated in the abdominal bodies of the rat by Hollinshead (143).



Continuous discharge over completely deafferented pupilloconstrictor neurons has been noted by Keller (144). Kuntz & Richins (145) suggest that adrenergic fibers promoting pupillary dilatation in cats arise in the ciliary ganglion; these are activated by impulses from the parasympathetic oculomotor center which in turn is activated by pain impulses from the periphery. If confirmed, this observation would obviate the necessity of assuming a central inhibitory mechanism in reflex pupil dilatation (146).

The sympathetic innervation of gastrointestinal blood vessels in rats includes both vasoconstrictor and vasodilator elements, which may be activated over simple segmental reflex arcs by warming and cooling of the skin (147). Distention of a jejunal Thiry fistula may produce decrease in tonus of the cardia in dogs (148). The paths of this reflex have not been worked out, but it is not intrinsic.

### CENTRAL MECHANISMS

Functional centers for pressor and depressor reflexes may be accurately localized in the medulla of the cat (149). The depressor center actively inhibits other cardiac centers. It is a curious fact that stimulation of one pressor center produces activity in the ipsilateral cervical sympathetic with reciprocal diminution in activity in the opposite trunk. Factors affecting the survival of the isolated respiratory center in rats have been studied by Hiestand and co-workers (150, 151).

*Hypothalamus.*—Analysis of hypothalamic functions is still being carried out by the methods of stimulation, application of drugs, partial transections, and localization of lesions. Refinements in experimental techniques and enlightened clinical observations may in time add much to our knowledge. It has been suggested, with some evidence, that hyperglycemia under general anesthesia results from release of the hypothalamus from cortical control (152). While a hypothalamic mechanism for release of epinephrine has been generally acknowledged, Rogoff *et al.* (153) suggest that a center for inhibition of such release is located in this general area.

There is evidence that the insecticide, DDT, when injected intravenously may cause marked sympathetic discharge through hypothalamic stimulation (154). Prolonged treatment with estro-

gens (156) may cause invasion of the neurohypophysis and even the hypothalamus with proliferated cells of the pars intermedia in hamsters; the supraoptic and paraventricular nuclei show degeneration. Repeated and finally fatal exposure of guinea pigs to low atmospheric pressures produced extensive degeneration of neurons in the hypothalamus as well as in other areas (157); a curious and perhaps significant finding in the light of Scharrer's work (158) was an accumulation of colloid-like material about cells of the supraoptic nucleus.

Ector's paper on hypothalamic pilomotor centers in the cat and monkey is now available (159). It is of interest that the descending paths are homolateral, and that epinephrine probably plays no part in physiological pilomotor activity. Stimulation of the hypothalamus has been reported to produce a neutrophile leucocytosis with markedly increased phagocytic activity (160). The latter was apparently due to release of an activating substance from the spleen.

An analysis of extensive experiments in stimulation of the hypothalamus by the method of Hess (161 to 164) has been made by Hess and co-workers (165). The method involves stimulation with implanted electrodes in unanesthetized unrestrained cats, using a damped pulsating direct current varying from 0.5 to 4 volts, at a frequency of eight per second. A possibility that such a current may cause tissue damage with related effects should not be overlooked (166), but many of the described reactions must have been due to stimulation. The positive responses observed were usually complete patterns involving vegetative activities and were often associated into coordinated attitudes and activities, i.e., protective emotional behavior, defecation, etc. There was also a peculiar negative response called "adynamia," elicited by weak stimuli from the lateral and anterior hypothalamus, manifested by reduced activity with great muscular weakness (167). It resembled sleep except for unnatural posture, and was reversed by strong stimuli. The externally visible coordinated responses were obtained mostly from longitudinally located zones, medial, intermediate, and lateral, rather than from specific points or antero-posterior areas. The medial area was relatively unreactive. The posterior hypothalamus seemed associated with sympathetic activity, while the rostral region and preoptic area seemed devoted

to complex mechanisms whereby activities of vegetative and somatic systems were synthesized into patterns; for instance, the coordinated somatovegetative act of defecation. Liquidation of the concept of topographically circumscribed hypothalamic centers is suggested. The most frequent responses correlated with region were: blood pressure increase with the posterior and intermediate zone; blood pressure fall with the lateral zone, anterior region, and septum; defecation and urination with the intermediate zone through the septum and hypothalamus, the coordinated act especially from the septal area; panting with the preoptic area, as an isolated heat regulatory factor; affective (rage) reactions with the intermediate hypothalamic zone; biting and avid eating (bulimia) with the intermediate zone with strong stimuli; coordinated sniffing and smelling of objects or air with the medial forebrain bundle (168). Hess believes these results in general agree with those of other workers, allowing for differences in techniques. Somewhat altering his previous views, he feels that the hypothalamus is a "dynamogenous" region, promoting synergism of vegetative and animal functions, a point of view which seems to the present writer to be a very useful one. An unexplained minor discrepancy is that parasympathetic responses were obtained especially from rostral regions, but not from the posterior region which their pathways must traverse.

In addition to their other findings, the Hess group observed that stimulation of the posterior hypothalamus seemed to promote motor initiative or drive and that stimulation here lowered the thresholds of centers for skeletal activity, also increasing the strength of response. It is interesting in this connection that Murphy & Gellhorn (169) found the motor responses elicited by cortical stimulation to be facilitated by simultaneous stimulation of the hypothalamus. While the hypothalamic areas involved were the same as those producing sympathetic effects, sympathetic discharges in themselves were not involved in the facilitation, which was presumed to take place at the cortical level. Rhines & Magoun (170) have obtained similar results with the additional observation that pallidal connections may play a role. The facilitatory area extended caudalward through the central gray, tegmentum, and bulbar reticular formation. These workers found that the motor facilitation must be consummated in the spinal

cord, because it takes place upon stimulation of the bulbar pyramid after extirpation of cortex. However, the hypothalamus may even so be capable of influencing cortical activity, since Murphy & Gellhorn observed that hypothalamic stimulation produced changes in the electrocorticogram. Cortical firing, elicited by local strychninization, was also increased in frequency by stimulation of the hypothalamus.

It should be pointed out, as a converse to the above, that lesions involving the caudal hypothalamus and pallidal connections have often been associated with hypokinesia.

Both inhibition and excitation of the colon have resulted from stimulation of a variety of nonspecific hypothalamic areas (171). The responses were in part mediated through the sacral outflow. No evidence of separate sympathetic and parasympathetic centers in the diencephalon was found. While hypothalamic stimulation will cause ovulation in rabbits, there are indications that this influence upon the anterior pituitary is not mediated by a direct nervous pathway (172).

It is evident that appetite and food consumption alone are not adequate to account for hypothalamic obesity (173, 174, 175). Changes in metabolism and activity are contributory.

Transient decrease in glucose tolerance has been observed to follow major transections of the brain stem and decerebellation as well as lesions of the tuber cinereum (176). A case of "central diabetes mellitus" with polyphagia and polydipsia was coincident with a saccular aneurysm in the third ventricle (177).

Disturbances in sleep mechanisms have been comprehensively reviewed by Davidson & Demuth (178, 179, 180). While emphasizing the importance of the cortex, the function of the hypothalamus as a regulatory (waking) center is reaffirmed. Nauta (181) has studied the hypothalamic regulation of sleep by producing partial or complete transections at various levels in rats. He confirmed the localization of a "waking" center in the caudal hypothalamus, but since transection in the preoptic area seemed to prevent sleep, he has postulated a "sleep center" in this region, the function of which is to produce sleep by inhibition of the waking center. Hess was an early proponent of the idea that sleep was an active process and could be caused by stimulation in the hypothalamus. It is difficult exactly to ascertain his present views from

his recent writings in regard to the "dynamogenous" hypothalamus, but he apparently still believes sleep can be produced by suitable stimulation (182).

Clinical records of disturbances in emotional balance or expression associated with hypothalamic lesions are increasing (183 to 187). Peculiar states of fury have been reported following operations or traumata involving this region. Paroxysmal disturbances of autonomic nature may also occur. Murphy & Gellhorn (188) have analyzed reciprocal corticothalamohypothalamic relationships by the method of strychninization and relate them to the problem of emotion. However, while there is good evidence that the hypothalamus may be concerned in emotional behavior, it is true that facio-vocal expressions of affective types may be evoked by nociceptive stimuli in cats with large transverse lesions at the mammillary level (189). These responses were abolished by central midbrain lesions, and this area must contain a mechanism for integrating this aspect of emotional expression.

It is reported that destruction of the suprachiasmatic nuclei abolishes sexual activity in male rats (190).

*Cortex.*—Autonomic disturbances have been reported to follow the operation of prefrontal leucotomy. These may include edema and vascular changes in the lower extremities (191, 192). Increased resistance to the effects of certain drugs such as prostigmine, ephedrine and amphetamine following this operation has given some indication of improved "autonomic equilibrium" (193). Autonomic effects of electroshock therapy have been studied (194). Vasodilator drugs have been reported to inhibit electrically induced convulsions in some cases (195), while prostigmine may lower the convulsive threshold (196).

Rats of certain emotional types have developed hypertension after repeated disturbance by an air blast (197). Significant relationships between autonomic and electroencephalographic changes during hyperventilation have been reported (198).

A cortical autonomic center for the eyes has been studied by stimulation technique (199). Both parasympathetic and sympathetic types of pupillary dilatation could be evoked. The area is located on the medial surface of the frontal lobe anterior to the cruciate sulcus in the cat.

## MISCELLANEOUS

The autonomic innervation of the inner ear has been studied (200, 201).

Kuntz has discussed lesions of autonomic ganglia from the standpoint of disease and aging (202). Safford & Gellhorn (203) have demonstrated experimentally that the reactivity of the sympathicoadrenal system diminishes with increasing age. Kuntz (204) has also reviewed the data relating autonomic nervous functions to allergy. The third edition of this author's valuable book on the autonomic nervous system has recently appeared (205). Nervous factors in the general adaptation syndrome have been discussed by Selye (206).

Bruesch & Richter (207) have presented evidence which indicates that the high skin resistance zones produced by nerve section may shrink because of ingrowth of fibers from adjacent intact nerves.

Again it may be emphasized that thinking of the sympathetic nervous system in terms of mass discharges and diffuse effects is not always in line with the facts. It has been shown that restricted, segmental sympathetic effects may be produced by stimulation of the spinal cord (208). Similar restricted, selective or area-specific effects may be elicited from the brain stem. It is no doubt true that when widespread discharges occur they must depend upon the simultaneous, sometimes integrated, activity of extensive central areas. We may thus add another item to the complex of paradoxes, contradictions and exceptions which give piquancy to contemplation of the autonomic system.

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DEPARTMENT OF ANATOMY  
STATE UNIVERSITY OF IOWA  
IOWA CITY, IOWA

## DIGESTIVE SYSTEM<sup>1</sup>

BY HARRY GREENGARD

*Department of Physiology, Northwestern University Medical School,  
Chicago, Illinois*

*General.*—The establishment after birth of a definitive propulsive motility pattern of the alimentary tract was investigated by Roufogalis (1), who noted in one hundred new-born children that peristalses are sluggish at birth, occurring only to the extent required to move bile and swallowed amniotic fluid to the distal intestine. Shortly after birth the rectal mucous plug is passed, and meconium is evacuated on the first day of life. There is a steady increase in gastrointestinal activity from the first to the fourth day, after which it remains essentially constant; the average values found for passage time were thirty-two hours on the first day, twenty-three hours on the second, ten hours on the third, and seven hours on the fourth. Weeks (2), observing the effects of various agencies on motor activity in the small and large intestine of a soldier with post-traumatic fistulas, found motility stimulated by food, excitement, intravenously injected hypertonic salt, spinal anesthesia, pitressin, prostigmine, and morphine; motility, however, was unaffected by locally applied heat or cold, calcium gluconate, or atropine.

The afferent nervous pathway responsible for the anorexia and vomiting produced by intestinal distension has been analyzed by Herrin & Meek (3). When a jejunal fistula is distended in the dog, these symptoms were not modified by bilateral vagotomy alone; the vomiting was abolished by bilateral splanchnicotomy and lumbar sympathectomy and a combination of the two procedures, i.e., complete extrinsic denervation, eliminated both nausea and anorexia. Anorexia resulting from intestinal distension may be due to impulses mediated by the vagi and sympathetics.

The effect of exsanguination on gastrointestinal motility has been found to be inhibition of gastric motility and stimulation of colonic activity, with apparently a gradient throughout the intervening gut between these extremes (4). This occurs in the non-narcotized as well as the narcotized dog, and is interpreted as the

<sup>1</sup> This review covers the literature from July, 1945 to July, 1946, with the exception of a few articles published since 1942 and not previously reviewed.

product of vascular influences, nervous influences, or both, since it is not encountered in animals shocked by other means to a comparable fall in blood pressure.

The barrier imposed by the gastrointestinal mucosa to the access of agents which stimulate its musculature has been demonstrated by Chiray *et al.* (6), who obtained violent and sustained contractions of the isolated stomach of guinea pigs sensitized to foreign serum when either the allergen or histamine were added to the saline bath in which it was suspended, but no response when the same amount of these substances was introduced in the lumen of the stomach. Similarly, Fergen & Campbell (7), who recorded the activity of isolated ileal strips from sensitized guinea pigs, found that the typical response to the antigen, to histamine, or to acetylcholine did not occur when the strips were everted. It has not been clarified how the mucosa acts to prevent the effective agent from acting.

Certain areas in the brain appear to stimulate various structures in the digestive tract. When acetylcholine, eserine, or prostigmine were injected into the hypothalamic region of the anesthetized cat by Emmelin & Jacobson (8), there was inhibition of jejunal motility, some suppression of gastric secretion, and stimulation of salivary secretion; in effect these are the product of sympathetic activity. When these agents were injected suboccipitally, there was stimulation of intestinal motility, indicating an action on vagal centers. (The innervation of the digestive tract is further considered in the chapter on the visceral functions of the nervous system in this volume.)

The fate of inert material in the alimentary tract has been the subject of several studies. The digestion of cellulose in man was examined by Chiray and collaborators (9); on the basis of determinations of ingested and fecal cellulose, the extent of digestion ranged from 20 to 100 per cent, the higher values obtaining in "soft" or finely divided cellulose. The human digestive tract contains no cellulose-splitting enzyme, and digestion is dependent upon bacterial action. The end products of digestion are alcohols and volatile acids, which are probably of minor significance as a source of energy in man and other species with the exception of ruminants, in which the pH change in passage from rumen to reticulum may stop the breakdown of cellulose at the stage of glucose.

A group of investigators at Cambridge, England, has examined



extensively the formation, absorption, and utilization of volatile fatty acids, particularly in ruminants. Marshall & Phillipson (10) and Elsdén (11, 12) identified acetic, propionic, and butyric acids as products of fermentation of cellulose, lactic acid, and glucose in the rumen contents from freshly killed sheep and sheep with rumen fistulas. The fatty acids were separated both chromatographically and by distillation. The rate of fermentation depended on the previous dietary history of the animal as the factor determining the presence of a favorable bacterial flora. Danielli and co-workers (13) introduced into rumen fistulas the sodium salts of these fatty acids and observed that at an alkaline reaction they were absorbed as their sodium salts, the absorbability varying inversely with molecular weight; whereas, at an acid reaction, the free fatty acids were absorbed, and absorbability varied directly with molecular weight. A tendency to the attainment of neutrality of the rumen contents by selective absorption was reported, and on the basis of the absorptive behavior, it was postulated that the salts are absorbed through water-filled pores in the intercellular cement, whereas the free acids traverse the lipid membrane of the cells. Elsdén *et al.* (14) analyzed the contents of the gastrointestinal tract at various levels in ruminants (sheep, ox, and red deer) and non-ruminants (horse, pig, rabbit, and rat). In the latter group the high concentration of volatile fatty acids in the rumen decreases rapidly through the other stomachs and the small intestine, increases sharply in the cecum, and then declines through the colon; a similar situation obtains in nonruminants, except that the concentration in the stomach is low. A definite relationship exists between fatty acid concentration and extent of fermentation; the fatty acids are a mixture of acetic (67 per cent), propionic (19 per cent), and butyric (14 per cent) in a quantity totalling 1 to 1.5 grams per kilogram of body weight. According to Barcroft (15), the general circulation in ruminants contains 0.4 to 0.9 millimols of volatile fatty acid per liter of blood, and the venous drainage from the large intestine up to 4.9 millimols per liter. In the sheep rumen, the rate of absorption is 2.3 to 5.3 gm. of volatile fatty acid per hour; introduced acetic acid disappears to the extent of 75 to 80 per cent in twenty-four hours and 95 per cent in forty-eight hours. Approximately 1 per cent of this amount is excreted in the urine; the remainder is destroyed.

The obvious desirability of attaining adequate penicillin medication by oral administration has prompted determinations of its

absorbability from the alimentary tract. Chu *et al.* (16) have demonstrated that it was absorbed from the esophagus, stomach, duodenum, and ileum of anesthetized cats, in each region to about the same extent, amounting to approximately one-fifth of the administered penicillin; it was also absorbed from the oral and nasal mucosa of dogs. McDermott *et al.* (17) observed that in the human subject absorption of penicillin, in considerable amounts, took place almost entirely in the small intestine; absorption was negligible from the stomach and colon. They believe that inactivation in the stomach is not extensive and does not depend on the gastric acidity, since it is essentially the same in normal and achlorhydric subjects, although there has been considerable evidence indicating complete inactivation at a pH below 3.0. In the colon, bacterial action produces extensive inactivation. The high dosage of penicillin required to produce an adequate blood level is considered to be made necessary by the fact that absorption is incomplete, rather than because of destruction, and the existence of any barrier offered by the liver is refuted by the findings that the penicillin blood level after injection into the portal vein is no less than when any other vein is the point of entry. The extent of absorption was determined as about one-third of the administered penicillin, and it was concluded that if a sufficient amount can be given orally, an adequate blood level is attainable. The use of "stabilizers" was decried, since the release of penicillin might take place too low in the gut. By following its effect on the circulation, Zachowski (18) noted paredrinol to be absorbed readily from the cat intestine, less rapidly from the mouth, and least from the stomach.

The capacity of malnourished and debilitated patients to ingest and utilize a very high-caloric, high-protein diet, given in the form of three large meals with interspersed hourly feedings, was demonstrated by Goodman & Garvin (19), who employed this regimen for correction of the deficient nutritive state of a large number of war casualties. The patients experienced no nausea or anorexia after the first day or so, and, in general, symptomatic improvement was marked in patients with a variety of war wounds, infectious hepatitis, malaria, psychoneurotic states, ulcerative colitis, and amebiasis. The characteristics of similarity between sprue and pernicious anemia have led Darby & Jones (20) and Spies and co-workers (21) to appraise the efficacy of folic acid in the treatment

of sprue, with reported marked symptomatic improvement. Folic acid is apparently effective in all macrocytic anemias.

#### MOUTH

*Teeth.*—In a caries-susceptible group of rats there was a high incidence of development of caries on diets high in glucose or sucrose in comparison with that occurring when the starch equivalent of these sugars was fed (22). Sugar caries did not occur in a caries-resistant group, and the difference was believed due to the fluorine content of the diet previously fed. The incorporation of vitamin K in chewing gum diminished significantly the incidence of caries in man, according to Burrill *et al.* (23), apparently by reducing oral acidity through its action as an enzyme inhibitor. The extension of existing caries was not modified. The degree of dental caries was found to bear no correlation to salivary amylase level (24), contrary to previous reports; and in periodontal disease, a significant increase is reported to obtain in the degree of putrefaction of the salivary proteins when saliva is incubated, as indicated by odor and by content of free amino acids, sulfides, and indole (25).

*Salivary glands.*—The viscosity of pilocarpine-stimulated salivary secretion is reported to be affected by variations in pulmonary ventilation (26). In exposure of human subjects to lowered oxygen tension, Lering (27) noted salivary flow to decrease and the pH to fall. Bråhammer & Emmelin (28) obtained inhibition of salivary secretion in response to chorda tympani stimulation or cholinergic drugs by a variety of barbiturates, whereas this was unaffected by xanthine derivatives which antagonize the effects of parasympathetic action on smooth muscle (29). Brassfield *et al.* (30) found the administration of ammonium hydroxide or carbonate to decrease the salivary pH while increasing blood pH, and to cause hypersecretion of the submaxillary gland in response to chorda tympani stimulation or cholinergic drugs.

Langenskiöld (31) recorded some 2500 electrograms from the submaxillary glands of forty-six decerebrated cats and four dogs, after stimulation of the chorda tympani and cervical sympathetic, by means of a string galvanometer and an amplifier which eliminated changes in resistance. Instead of many components, as had been previously reported, only two were seen when the current used was adequate to stimulate maximally, but was not too strong. The

relation between the two components varied according to the nerve stimulated; parasympathetic and sympathetic stimulation affected the same cells, and produced the same electrical components in different quantitative relation to each other. The two components were separable into a positive one directly paralleling the water and salt output of the gland, and a negative component paralleling the elaboration of organic matter and secretion only if superimposed on a positive one. An isolated positive component was obtainable after either prolonged or weak stimulation, and an isolated negative one was obtainable on stimulation after compression of the carotids or atropinization. Eisenbrandt (32) determined oxidation-reduction potentials on resting saliva in seven human subjects. The average value of 1,555 determinations was +301 mv.; values were very similar for the individual subjects at the same period of time under identical conditions, but group values manifested marked variations from month to month during a year's test period. The pH averaged 6.64. Krasnow (33) adapted to saliva the procedure for determination of phospholipids in blood, and considered low levels to be indicative of a healthy state.

#### ESOPHAGUS AND CARDIA

The recent literature has been reviewed by Benedict (34). The esophageal activity of two human subjects following dehydration was recorded optically by Ladell (35); activity increased for some time, depending on the severity of the dehydration, and disappeared after swallowing, with no other departures from normal. Pennington *et al.* (36) examined the relation of pressure changes in a jejunal Thiry fistula to tone changes in the cardia, and its relaxation following peristalses induced by swallowing or distension. Slight increases in pressure were ineffective, but repeated moderate distension caused some relaxation without nausea or distress; there was no effect on the typical relaxation-contraction pattern after peristalsis. In the dog, Rall *et al.* (37) stimulated the vagus nerve and observed, by means of recording levers and radiographic methods, that there was a contraction of the longitudinal fibers and a pulling up of the stomach toward the hiatus. This was considered a possible mechanism in some cases of hiatal hernia. Alvarez (38) has described a case of congenital absence of the cardia, which is of physiological interest in that it refutes the theory held by some that no true cardiac sphincter exists, and that the constricting action of the diaphragm is the effective barrier between the esophagus and

stomach. Alvarez's patient manifested distressing regurgitative symptoms, particularly in the recumbent position. Fleminger & Smith (39) cite a case of dysphagia due to achalasia at both the superior and inferior esophageal orifices following a depressed fracture of the skull with an extradural hematoma in the right cerebellar fossa; the dysphagia persisted after recovery and cleared up after octyl nitrite inhalation. Scott (40), who has characterized four clinical types of idiopathic esophageal dilatation, including achalasia, cardiospasm, constrictive, and dolichoesophagus, recommends treatment by subdiaphragmatic esophagogastrostomy if mechanical dilation fails, with immediate operation if dolichoesophagus were diagnosed. Hillemand *et al.* (41) have treated esophageal dilatation and cardiospasm by injection of the splanchnic nerves or splanchnicectomy; and Longmire & Ravitch (42) have devised an ingenious method for the construction of an artificial antethoracic esophagus from a loop of jejunum enclosed in a skin tube, severing the original pedicle after a new blood supply became established. After finding the procedure feasible in dogs, the investigators successfully applied this treatment to two patients with esophageal strictures and to one with atresia.

#### STOMACH

*Hunger and appetite.*—Meyer, Sorter & Necheles (43), on the basis of the objective presence of hunger contractions in patients with psychoneuroses and anorexia nervosa, have attributed the anorexia to a failure of the patient to perceive them. Treatment with amphetamine was found by these authors to improve the appetite, possibly by stimulating cerebral activity to the point at which hunger contractions reach a conscious level. Apparently the drug acts otherwise in the absence of mental instability, for Harris & Ivy (44) secured anorexia by the daily preprandial subcutaneous injection of *d*-amphetamine in a series of dogs maintained on a one-meal-a-day diet of constant composition fed every day at the same hour. The anorexia, the degree of which was dependent on the dosage, disappeared on the first day after discontinuing the drug, and the pattern was unmodified by vagotomy or splanchnicectomy, singly or combined.

*Gastric motility.*—No relationship between gastric emptying time and the fat content of the diet was found by Killian & Marsh (45), except for delay in the presence of excessive fat. A measurable delay in the emptying from the stomach of a test meal of

gruel and barium sulfate occurs, according to the observations of Henschel & Sturgeon (46) on eighteen human subjects, after a six-month period of semistarvation. Peterson & Peterson (47) have compared the inhibitory effect of dibutoline (dibutylurethane of dimethylethyl- $\beta$ -hydroxyethylammonium sulfate) and of atropine in 0.5 mg. doses. The atropine effect was more prolonged, but there were no side effects manifested with dibutoline when the dosage was less than 5 mg.; at higher dosages inhibition of motility was more prolonged. Northrup & Van Liere (48) have administered 10 grains of chloral hydrate to twelve human subjects prior to the ingestion of an opaque meal; in eleven of the twelve gastric emptying time was shortened, to a statistically significant extent in three, thus indicating a slight but measurable action of the drug in accelerating gastric evacuation.

*Electric potential.*—Bozler (49) has devised the equipment and technique adequate for the satisfactory recording of action potentials of the exposed stomach in animals, and has described three principal waves, designated *R*, *S*, and *T*. The duration of the complex was five to eight seconds, with rest periods of eight seconds duration interspersed. A single shock applied cephalad to the leads from the stomach at the end of the *T* wave elicited a premature peristaltic wave, followed by a compensatory pause. The potentials led off from the pyloric region were twice as great as in the case of the fundus, and greatest along the greater curvature. The *RT* interval was shortened by epinephrine, which in large doses was found to abolish all electrical activity. In reverse peristalsis, there was an inversion of all the waves. The form of the potentials is believed to indicate a syncytial arrangement of the muscle fibers. It has been reported by Rehm & Enelow (50) that a drop in the electrical potential of the stomach wall is produced by histamine, varying inversely with the secretory rate; when thiocyanate is administered in adequate but subtoxic doses, the secretory response is inhibited and the potential rises. The administration of further histamine in amounts adequate to overcome the thiocyanate inhibition produces again an increase in the secretory rate and a fall in potential. In these experiments thiocyanate was used in a dosage too low to affect the nonsecreting stomach. Measurements of the potential differences across the stomach wall by Rehm (51) have revealed that application of alcohol or ether to the serosal surface did not alter the value, in marked contradistinction to the decrease observed when the mucosal surface was subjected to their



action, indicating that the origin of the potential difference is in a more restricted zone in the stomach wall; this was located between the mucosa and submucosa.

*Gastric mucosa.*—Zucker and co-workers (52) have observed areas of epithelial hyperplasia in the fundus and rumen of rats on a deficient diet, and consider the fundic changes to arise from inanition, those in the rumen from protein deficiency. On a calcium-deficient diet lesions were reported (53) to develop in the mucosa of the antrum in the form of circumscribed, small, elevated areas showing hemorrhage, necrosis, and epithelial hyperplasia, which were aggravated by the administration of phosphate and diminished by vitamin D. Similar lesions were described as a result of a thiamine-deficient diet; curiously, they were abolished by giving calcium, though thiamine had no effect on the calcium-deficiency lesions. Jorpes & Thaning (54) have prepared from the mucosa of the abomasum of cows a polysaccharide which has a powerful neutralizing effect on the  $\alpha$ -agglutinins of Group B serum and on anti-A and anti-sheep hemolysins, being active in dilutions of 1 to 1,000,000. It was one-hundredth as potent in acting on the  $\beta$ -agglutinins of A serum. The material was not homogeneous, and by cataphoresis was separated into an acid fraction containing chondroitin sulfuric and mucoitin sulfuric acid, and a neutral polysaccharide, in a ratio of about one to four; the latter component consisted of approximately equal parts of acetylglucosamine and an unidentified sugar. Both fractions were of essentially the same potency. Dunham & Brunschwig (55) have failed to detect any changes, reflected in the gastric secretion of patients with cancer of the stomach, of the low calcium and high potassium content known to exist in rapidly growing neoplasms. The latter has received confirmation from analyses for these elements by Brunschwig, Dunham & Nichols (56), whose findings indicated that the carcinoma tissue in surgically-removed cancerous stomachs was low in calcium and high in potassium as compared with the adjacent uninvolved mucosa. One stomach contained a benign papilloma situated between two adjacent carcinomas, and this also manifested the calcium-potassium abnormality. Cox & Barnes (57) have made the interesting observation that some structural adaptation occurs in the hypersecreting stomach; they demonstrated a measurably greater number of parietal cells in the gastric mucosa of guinea pigs rendered hypersecreting by thrice-weekly histamine-beeswax mixture injections, in comparison to control



animals, indicating a hyperplasia arising from prolonged stimulation. Drobintseva (58), studying the behavior of cat mucosa in the Warburg apparatus, has observed increased activity of cytochrome oxidase during gastric secretion in response to food; at the height of secretion an increase in glutathione and ascorbic acid was reported.

*Gastric secretion.*—A new member of the family of theories for the formation of hydrochloric acid by the gastric mucosa has been fathered by Conway, Fitzgerald & Wallis (59), based on the assumption that an exchange occurs between isotonic potassium chloride secreted into the canaliculus of the parietal cells, and an organic acid formed within the cells. The result is postulated to be a passage of hydrogen ions out of the cell and potassium ions into it, coincident with which the organic acid anions are oxidized, leaving bicarbonate associated with potassium ions. This process is considered analogous to the formation of hydrochloric acid by yeast cells when suspended in an unbuffered medium containing potassium chloride. The pepsin content of gastric juice collected from the anesthetized cat has been determined by Bjorkman, Norden & Uvnäs (60); under continuous histamine stimulation it steadily declined to very low values, the rate of decline being dependent on the speed of histamine injection. At this point no increase in pepsin concentration results from an increase in the dose of histamine; a marked increase is produced by vagus stimulation, again reducible to negligible levels by sustained administration of histamine. These findings led the authors to assign no pepsin-stimulating properties to histamine, and to attribute the pepsin content of histamine-elicited juice to the amount present in the gastric glands at the time the drug is given. A rough but rapid method for pepsin estimation has been evolved by Kleiner (61), which is based on the clotting time of the mixture when buffered gastric juice is added to fresh milk. The more accurate hemoglobin method has been further adapted by Bucher *et al.* (62) in order to make it cover the activity of dog and human gastric juice over a wide range; the recommendation is made that dilution be high in order to minimize the action of pepsin inhibitors present. Bucher (63) has determined urinary pepsin output in a group of women; five of these, on a high protein diet, showed a twofold increase; four on a milk diet, no change; four on an orange juice diet, a 50 per cent reduction; and four on a high carbohydrate diet, a 50 to 67 per cent reduction.

Moersch, Rivers & Morlock (64) were unable to modify the

volume or acid output of histamine-stimulated gastric juice with a histamine inhibitor, benadryl ( $\beta$ -dimethylaminoethylbenzhydryl ether hydrochloride); likewise the secretory response to a meal was not inhibited, and the prospect of the utility of such a compound in the treatment of peptic ulcer was considered remote. Moreover, Sangster *et al.* (65) failed to note any inhibition of secretion from gastric pouch dogs under continuous histamine stimulation by either benadryl or by another histamine inhibitor, pyribenzamine (N'-pyridyl-N'-benzyl-N-dimethylethylenediamine). On the other hand, McElin & Horton (66) have described a tendency, not uniform, for benadryl to inhibit slightly the gastric secretory response in a series of patients receiving slow intravenous injections of histamine for the treatment of multiple sclerosis, when the dosage of histamine is small and that of benadryl large. Obviously, the balance of the evidence weighs heavily against the possibility of any practicability of such compounds in the role of antacids; although McGavack *et al.* (67) have reported some reduction in the acidity of the gastric contents (in response to an alcohol test meal) after three weeks of daily oral administration of benadryl to eight normal subjects and five patients with gastrointestinal neuroses.

Caravati (68) has presented evidence excluding both the possibility that the nausea and vomiting associated with salicylate poisoning is due to a local action of the drug on the gastric mucosa, which when examined gastroscopically manifested no abnormalities; and that they are due to its secretion in the gastric juice, which contained none regardless of how high the plasma level was. Kohn, Komorov & Shay (69) have injected neutral red intravenously in a dosage of 7.5 to 20 mg per kg. and observed it to operate as a weak stimulus to acid and pepsin secretion by the stomach, the response being reduced after vagotomy or atropinization, especially with regard to pepsin. None of the dye was excreted by the pyloric, mucous, or peptic cells, but appeared entirely in the secretion of the parietal cells, in which it may be stored in the resting stomach or when atropine is given. De Muro & Marconi (70), speculating on the gradual decline in secretory activity of the stomach with increasing age in the human adult, believe that they have obtained evidence of a specific hormonal effect of crude or purified testicular extracts in stimulating gastric secretion after parenteral injection. Acceptance of such a conclusion, on the basis of the evidence presented, imposes a heavy demand on the naivete of the reader.

Hartiata & Karvonen (71) exposed human subjects to the anoxia equivalent to an altitude of 18,000 feet or more, and noted a greatly reduced output of hydrochloric acid, unaltered by premedication with ammonium chloride. A marked inhibition of the gastric secretory response to histamine by the intravenous injection of histaminase has been reported by Rostorfer & Laskowski (72); in the dosage employed the animals manifested extreme nausea and prostration which constituted the mechanism involved in the inhibition of secretion, as attested by the finding that heat-inactivated histaminase manifested the same production.

The antacid effectiveness of an anion exchange resin (Amberlite IR-4) has been appraised by Martin & Wilkinson (73), who noted its action to be rapid and its neutralizing power considerable in the finely divided state. The material inactivated pepsin and trypsin extensively, but did not interfere with normal nutritive processes, as judged by the results of growth studies on rats; moreover, it did not affect the absorption of calcium or phosphate and was not constipating. Ogden & Southard (74) have investigated the gastric secretory patterns in human subjects after a meal of crackers and one of the following liquids: water, white wine, 14 per cent alcohol, dealcoholized wine, or acid tartrate solution. Wine elicited a secretion of more free acid than did water; the onset and termination of secretion were more delayed than in the case of alcohol, and dealcoholized wine and acid tartrate solution produced a small but sustained response. Roth & Ivy (75) have observed the gastric secretory response to caffeine to be diminished but not abolished by bilateral vagotomy in the cat, or by 1 mg. of atropine in the cat and man. On the basis of the evidence obtained, these investigators favor the view that caffeine acts directly on the parietal cells.

In dogs prepared with an intestinal fistula and a gastric pouch or fistula, Friedman, Pincus & Thomas (76) have reported that the instillation into the intestine of water, saline, or 0.1 N hydrochloric acid evoked an increased gastric secretion while a stimulus of food, histamine, or insulin was operative, but not otherwise. In each case the increase was related to the volume of fluid introduced. The response to food or insulin could be inhibited when the pH of the intestinal content was reduced artificially by dilute acid instillation to 2.5 or less, followed by a secondary stimulation which verifies a previous observation. Uvnäs (77) examined the relation of the pylorus to gastric secretion elicited by vagus stimulation, which he reported to be markedly diminished or abolished after occlu-

sion of either the arterial or venous blood supply to the pylorus, after cocainization of its mucous membrane, or after its extirpation. On the basis of these findings, it was postulated that vagus stimulation results in the elaboration from the pylorus of a humoral agent. This hypothesis is supported by the finding that certain pyloric extracts, which evoked but little gastric secretion on injection in the pylorotomized animal unless the vagus was concomitantly stimulated, produced a marked response during such stimulation. Histamine was not found present in such extracts.

Munch-Peterson, Rönnow & Uvnäs (78) have obtained a concentrate from the pyloric mucosa of cats and pigs which on injection is reported to elicit a strongly acid and pepsin-poor gastric secretion in the anesthetized cat. The extract did not depress blood pressure nor have any other histamine-like qualities, and was considered to be one with Edkins's gastrin. Uvnäs (79) subsequently has noted such a concentrate frequently to manifest a tachyphylaxis-like phenomenon on repeated injection; its ability to stimulate pepsin secretion was variable but slight and more apparent in the cruder concentrates. These findings suggest that a second principle stimulating pepsin may be present, having the same relation to gastrin as pancreozymin does to secretin. Active concentrates from human stomachs, obtained by gastrectomy or necropsy, have been prepared by Uvnäs (80). In a few instances, activity was noted in extracts of duodenal mucosa.

Investigation of the hormones inhibiting gastric secretion has proceeded apace. Friedman & Sandweiss (81) have devised a method intended to facilitate the always troublesome bioassay of such substances in which the ability of the material to inhibit secretion in the rat after pyloric obstruction is ascertained; at least twenty rats are required per assay. Kaulbersz *et al.* (82) prepared urogastrone concentrates from dogs which were normal, oophorectomized with and without thyroidectomy, or hypophysectomized, and have reported that urine concentrates prepared from the latter group have a stimulatory instead of inhibitory effect on histamine-stimulated gastric secretion. The gastric secretory responses of the various endocrine-deficient animals manifested tendencies to deviation from those of a normal animal, when the urogastrone concentrates from the groups were cross-tested (83). Way (84) has asserted that the hypochlorhydria of pregnancy is due to entero-gastric regurgitation, and that in pregnancy there appears to be an

inverse proportion between gastric acidity and the excretion of anterior pituitary-like substance in the urine.

Hollander, Stein & Lauber (85) have collected mucus from pouch dogs in an anacid (fasted and purged) state, and have examined some 500 samples of spontaneous secretion and secretion elicited by mild mechanical or chemical stimulation. The consistency varied from fluidity to jelly; the clarity from transparency to opacity; and the cellular content from none to many, including red and white blood cells and detritus as well as mucosal cells. These three properties were interrelated and dependent on the extent of irritation. They have postulated that either the neck chief cells and columnar cells are the source of mucus and mucoid secretion, respectively, or else that the cuboidal cells are the precursors of the columnar cells, and mucus comes from both; they suggest that its protective action depends on both secretion and surface epithelial desquamation. Hollander & Lauber (86) have reported the calcium content of mucus obtained by contact stimulation of gastric pouches with mild irritants to range from 2.7 to 13.3 mg. per 100 cc. In all cases the calcium content was less than the simultaneously determined serum calcium level; all specimens had a pH above 7.4, its value correlating poorly with the calcium content. Shay *et al.* (87) have observed a gastric mucigogue effect when sodium alkyl sulfate was instilled in the stomach of anesthetized dogs. This action was unaffected by atropine and considered purely local.

The previously-reported frequent occurrence of achlorhydria in rheumatoid arthritis has not been confirmed by Lucchesi & Lucchesi (88), who noted no abnormalities in the response of twenty-five such patients to an alcohol test meal. Helander (89) has extensively studied gastric secretion in fifty-one patients with *Bothriocephalus* infestation, and noted a variety of departures from the normal, none of which were characteristic. Shay *et al.* (90) have reported a continuous hypersecretion of gastric juice in thiamin-deficient rats when the deficiency was sufficient to cause a weight loss of 25 to 30 per cent; the weight loss itself was apparently not the factor concerned, since a similar loss, produced by underfeeding an adequate diet, was ineffective. In both groups ulceration occurred, although it was more pronounced in the thiamine deficient animals.

#### PEPTIC ULCER

The application of physiological principles to the problems of

causation and management of peptic ulcer has been emphasized with increasing force in recent years. A number of articles bearing on the etiology of ulcer have appeared; the factor of heredity in the form of a report by Riecker (91) of identical twins who developed the condition at about the same time, and whose father had a proven ulcer; the factor of diet and emotional stress in the observation by Mirault-Kretschmar (92) of a sharp rise in incidence during the war years of 1939 to 1944, with a predominance of fresh ulcers in individuals with no previous history. The importance of radical dietary changes is emphasized, and the observation made of an incidence of gastric ulcers sufficiently high to bring the ratio of gastric to duodenal to 1 to 1. The emotional factor has been stressed by Cox & Junnila (93), who have reported that, in 83 patients hospitalized for peptic ulcer and 78 for an anxiety neurosis, the two conditions co-existed in all 161. These authors suggested that the important factor in ulcer genesis is a play of impulses from a hyperactive central nervous system. Abrahamson (94) has been impressed, most unduly in the reviewer's opinion, by a suggestion that in ulcer patients the blood sugar levels are slightly below a level arbitrarily stated to be normal; and Ravault, Girard & Cessieux (95) by a frequent occurrence of polycythemia which to them suggested a relationship between peptic ulcer and overproduction of the intrinsic factor by the stomach, although the evidence presented requires a high degree of imagination for such a sweeping conclusion to be drawn. Hartman (96) has obtained duodenal ulceration (Curling's ulcer) in 78 per cent of dogs of which over 50 to 60 per cent of the body surface had been burned. All dogs showed positive blood cultures, and the incidence of ulcer was reduced to 24 per cent by penicillin therapy. A congested and edematous mucosa of the stomach and duodenum was present in the burned animals, and the susceptibility to corrosion by gastric juice engendered thereby is believed to be a potent factor in this type of ulceration. Iams & Horton (97) observed, among a series of patients receiving daily intravenous histamine for the treatment of multiple sclerosis, peptic ulcer symptoms in one patient who mistakenly reported for injections with an empty stomach, and in whom a gastric ulcer was demonstrated radiologically. The interpretation was based on the corrosive action of histamine-stimulated juice, unneutralized by food; the ulcer disappeared within ten days after histamine injections were stopped. Giddings *et al.* (98) failed in an attempt to produce caffeine ulcers in cats and rats;



understandably, because their experimental conditions were totally inadequate.

Bachrach *et al.* (99) have examined the evidence regarding the part played by autodigestion of the gastrointestinal mucosa in the genesis of peptic ulcer. None occurs in the stomach when circulation is adequate, when tolerance of the cells to acid and pepsin is not exceeded, and when the general condition of the subject is in a state adequate for mucus secretion, cell regeneration, and proliferation. In the intestine, sensitivity to acid and pepsin increases with increasing distance from the stomach.

Li & Freeman (100) have obtained ulcers in 47 per cent of dogs fed a protein-deficient diet in a minimum period of twelve weeks, especially when its fat content was low and bile salts were supplied. Shay *et al.* (101) have reported the production of ulcers in rats by pyloric ligation after a preliminary period of starvation; the lesions occurred uniformly in the rumen, less often in the antrum, and least in the corpus and were attributed to the constant presence of unneutralized gastric juice, since instillation with rat or human gastric juice or acid-pepsin solution produced the same effect. According to Driver (102), there is a definite prolongation of the time required to produce perforating ulcers by acid-pepsin instillation into isolated intestinal loops at low temperatures; and pre-treatment with mineral oil exerts a protective action from the corrosive action of such a solution (103). Driver & Carmichael (104) have reported that the addition of bile salts to the acid pepsin solution mitigated the damage produced.

Sandweiss *et al.* (105, 106), who have analyzed the nocturnal gastric secretion in a large series of normal subjects and in patients with uncomplicated duodenal ulcers, found that when it was collected by continuous aspiration, the volume and acid output were essentially the same in the two groups; however, the output was greater in the ulcer group when collections were made intermittently, which the authors attribute to delayed emptying rather than hypersecretion. They recommend that, if the patients' diets included such potent stimulators as meat or fish, these be fed at noon rather than in the evening, since acidity was higher in the latter case. Glenn (107), in a series of fractional analysis of histamine-stimulated secretion in two hundred ulcer patients and in two hundred and fifty nonulcer patients, including those with miscellaneous digestive disorders, has reported a greater average response in the ulcer group, with respect to the values at the height



of secretion, but with so much individual variation that the procedure is of no value diagnostically. Greenblatt & Cohn (108), investigating the cause of azotemia in bleeding ulcers, have described the findings in a group of normal subjects who drank 580 or 800 cc. of their own blood; there was a definite increase in blood urea, marked in the 800 cc. group, and reduction in urea clearance values. This they attribute to an interference with kidney function due to fluid loss; however, it has been previously reported by Schiff *et al.* (109) that azotemia is obtainable from ingestion of a comparable amount of blood or its protein equivalent, with or without preliminary bleeding.

Cummins *et al.* (110) have determined the healing time of peptic ulcer, based on roentgenological and gastroscopic disappearance of the crater as an objective measure, on uniform standard medical management in order to provide a criterion for new methods of treatment. In sixty-three duodenal ulcers it averaged thirty-seven days, and in six gastric lesions forty-two days, observations which check well with the observed healing time of experimentally produced acute ulcers in animals. There was no correlation with size of the crater, duration of symptoms, the age of the patient, or subsequent recurrences. The problem of recurrences has been analyzed by Raimondi & Collen (111), who followed 151 cases of proven peptic ulcer for from twelve to thirty-three months of conventional medical management and noted an incidence of recurrences of 5 per cent a month, 67 per cent in the first year, and 83 per cent in two years. They have concluded that the problem of prevention of recurrences is much more difficult than that of relieving the acute symptoms.

A number of adjuvant measures for the medical management of peptic ulcers have been advanced. CoTui *et al.* (112) have reported prompt relief of distress together with improvement in the status of the ulcer and general condition in twenty-six cases following the administration of protein hydrolysate with Dextrimaltose, accompanied by a decreased acidity of the gastric contents and a definitely positive nitrogen balance. There was no protection against recurrences if the post treatment course was uncontrolled. Vinci *et al.* (113) have also described favorable results in thirty patients on a similar regime. Nasio (114) has obtained protection against cinchophen-induced ulcers in seven of twelve dogs by the parenteral administration of 5 gm. of calciferol (vitamin D<sub>2</sub>), and feels that it arises from the hyposecretion secondary to hypercal-

cemia. Parrot, Debray & Richet (115) have noted the blood histamine level in peptic ulcer patients to be nine times as high as that of normals, and consider this to be the basis for hypersecretion; in eleven patients treated with Antergan, N- $\beta$ -dimethylaminoethyl-N-benzylaniline, a histamine antagonist, symptomatic relief was stated to occur in ten.

The most promising advance in ulcer therapy appears to be the lasting protection against recurrences afforded by concentrates from the intestinal mucosa and urine; the literature on this subject has been reviewed by Sandweiss (116, 117) who, impressed by the significance of the problem, pleads for a coordination of effort on the part of the various investigators in this field. Morrison (118) has reported complete prevention of cinchophen-induced gastric ulcers in dogs by the feeding of a poorly characterized concentrate from hog stomach and intestine, and believes that he has noted evidence of improvement in ten peptic ulcer patients who were fed 15 cc. of histamine-elicited gastric juice from normal individuals every hour (119). It might be legitimately concluded from his studies that the dosage was too low to aggravate the existing ulcer. Hemmeler (120) has reported healing in twelve cases of peptic ulcer treated by the intramuscular injection of a gastrointestinal extract designated Robudan. Ivy (121) has reviewed the findings derived from extensive studies on Mann-Williamson dogs and peptic ulcer patients concerning the lasting protection afforded by parenteral or oral administration of concentrates from an acid extract of intestinal mucosa; while Grossman & Ivy (122) have reported a protective action against ulceration in fourteen of seventeen Mann-Williamson dogs when desiccated intestinal mucosal extract was fed daily in adequate doses. The material effective by parenteral injection in the Mann-Williamson dog failed to protect against the development of ulcers induced by histamine-beeswax injections (123).

The surgical treatment of peptic ulcer has likewise been the concern of a number of investigators, who have interested themselves in the development of new techniques as well as the perfection of the more classical procedures. Dragstedt (124) has summarized his results following vagotomy on thirty-nine patients, describing uniform and lasting improvement, with weight gain and x-ray evidence of healing. This he has attributed to a correction of the interdigestive hypersecretion assumed to be the chief gastric secretory abnormality in peptic ulcer. But such an explana-

tion would appear to be irreconcilable with the findings of Sandweiss (106), cited previously, and likewise with Dragstedt's observation that after vagotomy gastric tone and motility were decreased, which would operate to favor the retention designated by Sandweiss as the basis for the increased quantity of juice collected intermittently in ulcer patients. Moore *et al.* (125) also have reported a very favorable course in fifteen ulcer patients subjected to transthoracic resection of a sizable portion of the vagi. In the hands of Baronofsky *et al.* (126) vagotomy failed to protect thirty-three dogs, six cats, and eight rabbits against the development of ulcerations induced by histamine-beeswax injections; moreover, three of six rabbits subjected to the procedure alone without histamine developed ulcers. In summing up the current status of this form of treatment, it may be stated that, though the present results are encouraging, considerable reserve should be manifested in accepting as desirable any therapeutic procedure involving destructive surgery. Somervell (127) has described a "physiological gastrectomy" involving ligation of most of the arterial supply of the stomach, which is stated to produce a marked reduction in gastric acidity. He reported only one recurrence in four hundred cases treated by this procedure in combination with gastroenterostomy in contradistinction to seven in three hundred subtotal gastrectomies. Wu (128) found that total thyroidectomy did not prevent the development of ulcers in Mann-Williamson dogs. Saltzstein & Kurtz (129) have applied additional fuel to the funeral pyre of the allegation that a jejunal pedicle graft constitutes a protective measure against peptic ulcer; in six Mann-Williamson dogs prepared with such grafts, five developed the typical jejunal ulcer, and the sixth, an ulcer of the graft.

Relevant to sequelae of the more conventional surgical procedures of gastroenterostomy and subtotal gastrectomy, Tosseland & MacDonald (130) have examined 100 cases in which gastric resections were performed for gastrojejunal ulcer and found the location to be on the jejunal side in 81 per cent, on the gastric side in 3 per cent, at the anastomotic site in 3 per cent, and undeterminable in the remainder. In most cases there were parietal cells adjacent to the anastomosis on the stomach side and occasionally Brunner's glands on the jejunal side. Attention has been drawn by Custer, Butt & Waugh (131) and by Berkman & Heck (132) to the postprandial distress occurring in 5 to 12 per cent of subtotal gastrectomies and designated the "dumping stomach" syndrome,

characterized by nausea, weakness, cold sweat, and palpitation, which both groups of investigators attribute to reflexes arising from distension due to the rapid passage of food into the unprepared jejunum. A narrow anastomotic stoma for prophylaxis, and a high-protein high-caloric diet for treatment are recommended. Wollaeger *et al.* (133) have found no steatorrhea or azotorrhea in seven duodenal ulcer patients or six normal subjects on a high fat diet; however, they have observed (134) wastage of fat, and in some cases of nitrogen, in fourteen patients who had undergone subtotal gastrectomy with gastrojejunostomy, and indicate the importance of a sufficiently nutritive diet to prevent weight loss.

#### PANCREAS

Elman & Hatch (135) have reviewed the recent literature. The ultra-violet absorption spectrograms of the exocrine cells of the pancreas have been analyzed by De Robertis and co-workers (136, 137). Their analysis showed that the basal portion of the acinar cells contains a substance with a maximum absorption at 2600 Å, identified as a nucleotide of the ribonuclease type, and the apical part gives the characteristic absorption curve of a protein, with maxima at 2400 and 2800 Å, indicative of a high concentration of protein in the granules. Treatment of pancreatic tissue with glycerol results in solution of the granules and elimination of the latter pattern. Reinhoff & Pickrell (138) have examined 250 autopsy specimens in order to determine the relation of the pancreatic duct systems to pancreatitis. In seventy-three, or 29 per cent, the pancreatic and biliary systems were entirely separate; in ninety-two, or 37 per cent, there was contiguity and coalescence of the pancreatic and bile ducts 1 to 2 cm. from the orifice; in eighty-one, or 32 per cent, a true ampulla existed, ranging in length from from 3 to 14 mm.; and in four, or 1.6 per cent, the pancreatic duct was reduced to a fibrous cord. In forty-seven cases, or 18 per cent, the ampulla was longer than the diameter of the common orifice. Thus, a block at the papilla would result in a communication between the bile and pancreatic duct. In eighty-nine of one hundred specimens, the main and accessory pancreatic duct systems were seen to communicate. Richins (139) has studied extensively the innervation of the cat pancreas, and observed all the nerves to pass through the celiac plexus and to reach the gland along the adventitia of its blood vessels. Just peripheral to the celiac ganglia the fiber counts average 3535 myelinated and 67,496 unmye-

linated, a ratio of one to nineteen; most of the latter are sympathetic postganglionic fibers, not significantly decreased in number by either bilateral vagotomy or splanchnicectomy. Most of the sympathetic preganglionic fibers terminate in the celiac ganglia; the parasympathetic preganglionic fibers end in the intrinsic pancreatic ganglia. Sympathetic postganglionic fibers travel solely to the blood vessels within the gland; the parasympathetic postganglionic fibers accompany the blood vessels between the lobules and acini, and send off fine twigs with many knob-like processes which must represent points of contact with the acinar and the islet cells; they innervate such smooth muscle as exists around the ducts. The sensory end organs are Pacinian corpuscles, the fibers from which run with the splanchnic nerves. Hermann *et al.* (140) have noted that the pancreatic secretory response to both vagus and splanchnic stimulation is potentiated by physostigmine and inhibited by atropine.

Harper & Mackay (141) have examined biopsy specimens of cat pancreas taken without treatment and following injections of secretin and pancreozymin. In the untreated animals, the zymogen granule content of the acinar cells was quite variable, being unchanged by secretin, and being markedly diminished, but never exhausted, by sustained vagus stimulation or repeated pancreozymin injections. Doubilet (142) has obtained confirmation of the potency and purity of crystalline secretin and separation of secretin and cholecystokinin according to the Greengard-Ivy process; the yield of cholecystokinin was very low. The potency of injected secretin was enhanced by the injection of vitamin K, probably on the basis of its inhibition of blood secretinase; it was shown to exert such an effect *in vitro*. Sheline, Chaikoff & Montgomery (143) have injected dogs with radioactive cobalt; a considerable amount (5 per cent) was excreted in the bile, and only 0.3 per cent in the pancreatic juice, during the ensuing seventy-two hours. Munro & Thomas (144) have subjected pancreatic juice, collected under the stimuli of peptone, soap, or dilute acid instillation and secretin injection, to electrophoretic analysis in a Tiselius apparatus, and noted, when a sodium bicarbonate buffer was used, four or five components, independent of the stimulus or the protein content; with a sodium diethylbarbiturate buffer an additional boundary usually appeared. Chaikoff, Entenman & Montgomery (145) have noted a low plasma choline level in dogs developing fatty livers subsequent to exclusion of pancreatic juice

from the intestine. Restoration of the plasma choline level and prevention or disappearance of the abnormal fat content of the liver may be effected, according to these investigators, by the daily feeding of pancreas concentrates in amounts as low as 60 mg. in the case of active extracts. The action appears to depend on either the mobilization of preformed choline or a synthesis of it from its precursors, since it occurs no less readily on diets very low in choline.

Laboratory diagnosis of pancreatic disease has been further developed. Blaubaum (146), studying two patients with fibrocystic disease of the pancreas, has obtained confirmatory evidence regarding the diagnostic value of intubation and aspiration of the duodenal contents and enzyme analyses, and the therapeutic merit of oral administration of pancreas extract. Lagerlof (147) has devised a test of pancreatic function based on measurement of serum amylase after the injection of secretin with morphine, the purpose of the latter drug being to block the ducts temporarily. Sjöberg (148) has evaluated pancreatic function in twenty-six patients, ten of whom were known to have pancreatic disease, by the methods of stool analysis, blood and urinary amylase levels, and the secretin test, and has drawn the conclusion that stool analysis is the most reliable. The secretin test was stated to be indicative only of quite severe deficiencies, and the amylase tests were consistently negative. Polowe (149), in studies on the blood amylase in sixty-nine cases, has reported the increased level found in thirty-two to be invariably associated with pancreatitis, which condition never existed in the presence of normal or low values; the latter may be associated with liver deficiency. It has been pointed out by Carter (150) that acute abdominal distress in known alcoholics may be accompanied by symptoms too severe to be gastritis, in which event pancreatitis should be suspected; in eleven such patients an elevated serum amylase level was found, and in four who were operated there was edema of the pancreas and a serosanguineous fluid in the peritoneal cavity.

Subtotal pancreatectomy for the relief of neoplastic obstruction to the common bile duct has been attempted on an increasing number of cases. The results of more than fifty cases of radical pancreatectomy in man have been reviewed by Orr (151), who has concluded that, despite the present high mortality, this procedure offers the best chance for a cure. Orr believes that good health is possible without the external pancreatic secretion, but recommends



union of the severed uncinat process of the pancreas to the gut. A technique for the latter procedure has been devised on dogs by Smithy, Pratt-Thomas & Mace (152), in which the pancreatic stump is anastomosed to the jejunum by an incision in the latter extending to, but not through, the mucosa, with establishment of drainage of pancreatic juice into the gut in 70 per cent of the animals, as verified by observation after secretin injection, and with no peritonitis or external fistula, even when a pancreaticojejunal fistula failed to develop. A method applicable to man has been developed by Varco (153), who employed a pancreaticojejunostomy by way of a rubber catheter implanted in the pancreatic duct and passed through the wall of the jejunum, with suturing of the jejunal mucosa to the pancreatic capsule. The catheter was passed after about ten days, and no digestive difficulties were encountered. Cole & Reynolds (154), have treated, with one fatality, five cases of carcinoma of the head of the pancreas with biliary obstruction by a resection of the duodenum plus the head of the pancreas. Dixon *et al.* (155) have described a case of total pancreatectomy in man for the treatment of carcinoma; postoperatively, there was fecal wastage of about half the ingested fat and a third of the protein, but no significant loss of calcium and phosphorus. The daily administration of 15 gm. of concentrated pancreatin reduced wastage by half; only twenty-five to forty units of insulin were required daily.

#### SMALL INTESTINE

The recent literature has been reviewed by Kiefer (156). Bozler (157) has obtained amplified oscillographic records of the differential action potentials of strips of small intestine from rabbits and guinea pigs, correlated with motor activity, and has identified an *R* and a *T* wave separated by a spike-bearing isoelectric interval, with both waves of fairly long duration; in contradistinction to the stomach (49) there was no *S* wave. The monophasic action potentials obtained by direct recording checked well with those calculated from the differential potentials. Each action potential was followed by a contraction; but marked variations in contractility were associated with only slight changes in potential. The electrical behavior was inhibited by large doses of epinephrine. In the guinea pig gut, the potentials were limited to spikes, the frequency of which was related to the magnitude of contraction. Youmans & Foltz (158) have obtained simultaneous record of pressure changes and oscillographically recorded alterations in potential in intestinal



segments. An increase in pressure was preceded by a slow smooth potential change, and was accompanied by numerous medium-fast potentials; the latter were accentuated by cholinergic drugs and eliminated by atropine or epinephrine, whereas the former persisted in modified form. Intestinal distension resulted in the appearance of numerous very rapid potentials, which decreased in frequency as the distension was relieved. It has been reported by Cheng, Hsin & Hsu (159) that in dogs and rabbits the cholinesterase activity is higher in the ileum than the duodenum, and higher in the muscularis than the mucosa. Acetylcholine was extractable from both muscularis and mucosa, and in larger quantities from the upper than the lower intestine. In the atropinized, vagotomized, and physostigminized rabbit, the jejunum was found to synthesize 24.5 per cent of acetylcholine in ten minutes.

*Intestinal motility.*—Ambache (160) has studied the behavior of intestinal strips after cooling to 0–2° C., the effect of which is to abolish acetylcholine synthesis and thereby provide a means for distinguishing drugs which stimulate smooth muscle through acetylcholine release from those which act directly on the muscle, on the basis of: (a) ineffectiveness after cooling; (b) potentiation after physostigmine; (c) inhibition by calcium and magnesium; and (d) potentiation during the calcium after-effect. The first change noted after cooling was a loss of peristalsis, and after four to five days the pendulum movements disappeared. Such a segment of gut was found responsive to acetylcholine or electrical stimulation but not to potassium, barium, or histamine; thus the action of these occurs through release of acetylcholine. The effect of all the drugs tested which operate through acetylcholine release could be abolished by calcium. Van Liere *et al.* (161) have determined the passage time of a charcoal-acacia mixture given by stomach tube to dogs which were sacrificed at intervals thereafter, and noted rapid progress in the first fifteen minutes, indicating a greater peristaltic activity in the upper intestine. Furchgott & Schorr (162) have investigated the ability of a number of substances, particularly a series of fatty acids, to provide energy for the contraction of rabbit intestinal segments; all fatty acids tested except propionic were capable of providing energy for muscular contraction, with a behavior in keeping with  $\beta$ -oxidation. McClendon & Scott (163) have investigated the motility of intestinal segments, isolated or *in situ*, after the introduction of acetic and butyric acids as such or as their sodium salts. When the salts were

so introduced, or injected intravenously at physiological pH at a rate more rapid than they could be metabolized, a stimulation of rhythmic contractions of the gut resulted. With a tandem-balloon system operating, the introduction of acetate or butyrate into the duodenum stimulated contractions successively downward as recorded by the two segments, indicating a distally directed passage of the wave of excitement. Motility was found to be depressed by the free acids, due to the pH effect; it was also abolished in the longitudinal direction by longitudinal section or eversion, due to release of tension, and restored by suturing. Learner *et al.* (164) have noted the intravenous injection of hydrolyzed protein to increase the tone and amplitude of contraction of intestinal musculature in the anesthetized dog, independently of vagal innervation and unaccompanied by a significant change in plasma pH, carbon dioxide, chloride, or total base, and an attendant hyperglycemia which was also atropine-resistant. The hypermotility did not damage suture lines recently established in the gut. The unanesthetized human subject was noted to manifest alterations in the normal contraction pattern and tone changes, correlated with symptoms in only about half of the cases. According to Hsu (165), the vagi carry fibers inhibitory to the intestinal musculature; he has reported that frequently vagus stimulation in the anesthetized dog causes a repression of intestinal motility, not antagonized by large doses of atropine, somewhat increased by physostigmine and mimicked by injection of acetylcholine into the mesenteric artery. Hsu & Yang (166) have reported that acetylcholine, like nicotine, produces a diphasic motor response of the dog intestine *in situ*; the excitatory phase is abolished by atropine, the inhibitory one only by large doses of nicotine; mecholyl acts similarly to acetylcholine, whereas pilocarpine and barium chloride are predominantly excitatory. The action is presumed to reside in the enteric ganglion cells. Caujolle & Franck (167) have noted that the essential oil of Marjolaine causes a marked stimulation of intestinal peristalsis when given orally or injected intravenously in alcoholic solution, the action being immediate in the latter case and after about an hour's delay in the former. Fractional distillation revealed the action to reside in the terpene fraction, with none in the terpene-free residue. According to the report of Caujolle, Franck & Grandpierre (168), calcium chloride inhibits intestinal motility temporarily, possibly by a direct action, and this is followed by increased peristalses, presumptively on a reflex basis. Brunand & Germain (169) studied

the response of rat intestinal strips in a saline bath to epinephrine and ephedrine as influenced by a sympatholytic compound designated 933F, which they reported to block quite effectively the inhibitory action of both drugs on the gut; when given alone it also depressed the tone and motility. In the same manner ephedrine was reported to inhibit epinephrine. The graphic records supplied suggest that the second agent was, in all cases, added while the inhibitory effect of the first was still operative. Bastenie (170) has reported that in twenty-nine patients with severe hypothyroidism there were disorders of intestinal motility ranging from simple constipation to megacolon, and from abdominal distension to paralytic ileus.

*Intestinal secretion.*—Gavrilov (171) has reported an increased excretion of sugar in the intestinal juice in chronic diarrhea. Valette & Cavier (172, 173) have noted the composition of the secretion of an obstructed and denervated jejunal loop to be that of a transudate of blood plasma, with the important exception that it was practically sugar-free whether or not the animal received insulin.

*Intestinal absorption.*—Follansbee (174) has fed anesthetized and unanesthetized rats 2 per cent of their body weight of water dyed with Evans blue, and has determined the osmotic activity and chloride content of samples from the stomach, duodenum, and jejunum. The osmotic activity was found to increase to isotonicity in relation to downward passage, and was higher in the unanesthetized group, where the length of intestine traversed was greater; occasional findings of hypertonicity were shown to be the product of bacterial activity. It has been reported by Goldberg & Fine (175) that bleeding morphinized dogs to a shock state causes a marked delay in absorption of water or glucose solution, with incomplete recovery after transfusion; absorption of normal saline solution was not affected until shock was extreme. Fenton (176) has noted that in rats fed glucose solution in concentrations varying from 5 per cent to 65 per cent, absorption was negligible from the stomach, and from the intestine it varied directly with the glucose concentration. With high concentrations, there was inhibition of both motor and secretory activity of the stomach, undoubtedly through invoking the animal's enterogastrone mechanism. Birchall, Fenton & Pierce (177) have fed rats glucose solutions in varying concentrations and volumes, and noted that a given amount of glucose was more rapidly emptied from the stomach when in dilute than when in concentrated solution, and more rapidly absorbed from concen-

trated solution. At a constant concentration, emptying and absorption were more rapid from large than from small volumes; at constant volume, from concentrated than from dilute solutions. The plasma vitamin A curve after the administration of a large amount (75,000 units) showed a small but significant decrease in its absorption when given with an ounce of aluminum hydroxide, according to Hoffman & Dyniewicz (178). This was not observed in normal subjects given alumina gel every two hours for two weeks beforehand, or in peptic ulcer patients on this form of management, and aluminum phosphate does not affect vitamin A absorption. It has also been reported by these authors (179) that alumina gel does not decrease absorption of neutral fat; there was a small and statistically insignificant decrease for amino acids, glucose, and vitamin C. Frazer (180) has observed the presence of large fat globules in the intestinal cells of rats fed 1 cc. of olive oil with 1 cc. of water; very little fat appeared to pass into the areolar tissue of the villi or the lacteals. The addition of choline chloride in a concentration of 0.5 per cent resulted in the appearance of masses of fat in the areolar tissue, while the cells were more rapidly cleared, and the fat remaining in them was more finely dispersed. The effect was immediate, and no evidence was obtained of more rapid gastric emptying or marked increase in intestinal motility with the 21.5 mg. of choline used. Cooke and co-workers (181) have performed fecal fat studies in 120 healthy and diseased subjects on a dietary fat intake of 50 gm. Normally, less than 5 per cent of the ingested fat was recoverable; there was increased wastage in idiopathic steatorrhea, sprue, pancreatitis, lymphatic obstruction, fistulous short-circuitings, and malnutrition. In all groups, 70 to 85 per cent of the ingested fat was hydrolyzed in its passage through the gut. It was concluded that neither determination of fecal fat or of degree of hydrolysis are diagnostic signs in disease. It was noted that in cases of defective absorption, 70 to 80 per cent of the fat can be taken up from the gut without lipemia; this also happens in normal subjects given fat with added lipase. The literature on absorption of triglyceride fat from the intestine has been reviewed by Frazer (182).

The observations of Visscher & Roepke (183) have provided evidence that when the osmotic activity of plasma is artificially raised by the intravenous injection of hypertonic salt, there is a rise in osmotic pressure of the ileal contents, but this was proportionately less. Water absorption from the ileum was not in-

creased in proportion to the increase in differential of osmotic pressure. Visscher, Roepke & Lifson (184) have reported absorption of water and chloride from autogenous serum placed in ileal loops of anesthetized dogs, despite absence of any concentration gradient, with a loss of sodium amounting to about 30 per cent that of chloride, and maintenance of ionic balance by diffusion of bicarbonate into the gut. There was a net loss of inorganic electrolyte corresponding to the fall in osmotic pressure, and the results were in accord with previous observations using labelled ions, in which it was noted that passage of water and salts occurs in both directions between gut and blood; the latter have a much greater tendency to pass from gut to blood than the reverse. The evidence re-emphasizes the complexities of intestinal absorption, particularly with the implication that pure water might be secreted into the gut.

#### COLON

Gauss (185) has apparently been exceedingly impressed with the importance of bile in the colon in maintaining an adequate water content of the fecal mass, and recommends the use of bile salts as a panacea for constipation. Brigham (186) has reported production of malnutrition in rabbits through limiting colonic motility artificially by shortening the mesocolon. Hoekstra & Steggerda (187) have found that pyribenzamine, although in itself causing some increase in tone and activity of the thorotrast-visualized colon of the dog, would in adequate doses inhibit histamine-induced contractions; the amount required approached toxic levels.

Ingersoll & Jones (188) have observed both excitatory and inhibitory effects on the colon from faradic stimulation at various levels in the thalamus, hypothalamus, and medially located structures of the telencephalon; abolished by cord section at L6 in over half the animals and diminished or modified in the rest, these effects are indicative of action through the sympathetics. No sharply defined centers were found in the region stimulated.

An attempt has been made by Rasberry (189) to minimize the distress due to intestinal gas after exposure to high altitude by altering the intestinal flora, in the direction of reduction of gas-forming bacteria, by succinylsulfathiazole administration. There was no change in visualizable intestinal gas at ground level; at altitude the subjects were more comfortable, probably due to increased ease of expulsion. Three healthy children with colostomies

have been observed by Friedman & Snape (190); the colonic stomal mucosa was noted to blanch after pain or other unpleasant stimuli and during expulsion of feces; there was reddening associated with the sight, smell, and ingestion of appetizing food, and after completion of fecal expulsion.

Dunham, Nichols & Brunschwig (191) have made determinations of the calcium and potassium content in carcinomas and papillomas of the colon, and found a low-calcium, high-potassium level in both the benign and malignant lesions; in the papillomas the calcium depression was less marked. In dogs prepared as in the Mann-Williamson operation, except for diversion via the duodenal stump of bile and pancreatic juice into the appendix, Ivy & Clarke (192) have reported all animals to die of perforated jejunal ulcer. None developed ulcerative colitis in accordance with the expectancy of corrosion of the colonic mucosa.

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DEPARTMENT OF PHYSIOLOGY  
NORTHWESTERN UNIVERSITY MEDICAL SCHOOL  
CHICAGO, ILLINOIS

## KIDNEY\*

BY WILLIAM DOCK

*Department of Medicine, Long Island College of Medicine, Brooklyn, New York*

### THE MEASUREMENT OF RATES OF EXCRETION OF SOLUTES

Much new work and several lengthy discussions deal with the determination and significance of the ratio of the amount of solute excreted in the urine in one minute to the amount in one cubic centimeter of plasma. Such a ratio<sup>1</sup> has been referred to as a clearance—that is, the imaginary volume of blood cleared of the solute in forming the urine passed in one minute. Ratios of the order of magnitude of that for creatinine presumably approximate the figure for the volume of glomerular filtrate formed per minute. Of certain solutes, such as diodrast, it is known that the blood in the renal vein contains less than 15 per cent of the concentration of that in the renal artery. The ratios for such solutes must approximate the minute flow of plasma through the kidney.

Under abnormal conditions such ratios may deviate from the true volume of glomerular filtrate (GF) or plasma flow (PF), and it would be more precise to record the values simply as urea, creatinine, diodrast or other "ratios." The true renal blood flow may now be measured by catheterizing the renal vein, and measuring arteriovenous differences in concentration of a solute while determining the rate of its excretion.

The maximal possible rate of tubular excretion or reabsorption of a solute,  $T_m$ , is determined only when the plasma level is very high. It is the difference between the amount of solute in the urine

\* This is a selective review of the reports which became available in the United States after July 1, 1945. It includes material published up to July, 1946.

<sup>1</sup> Editor's Note: The author of this review feels that the term "ratio" is to be preferred to the more conventional term "clearance" in stating rates of excretion of solutes by the kidney. While the use of "ratio" in this application is not in strict accord with the mathematical definition of the term, it might be justified on the ground of current English usage and in the interest of precise description. In the conviction that final adoption of preferred terminology should be determined by discussion and agreement among active workers in the field concerned, the editors call attention to the use of "ratio" where the term "clearance" has been employed in previous volumes of the ANNUAL REVIEW OF PHYSIOLOGY and in most of the current literature.

formed in one minute, and the amount in 1 ml. of plasma multiplied by the volume of glomerular filtrate per minute.

*The normal range of excretory ratios.*—Chasis *et al.* (1), carrying on the work on aromatic acids as substitutes for diodrast (2), describe in detail the use of *p*-aminohippuric acid, or the sodium salt in man. The ratio (clearance or PF) is  $621 \pm 133$  ml. per min. per 1.73 sq.m. of body surface (average adult size). Tm for *p*-aminohippuric acid is  $77.5 \pm 12.9$  mg. per minute in the average adult. The molar ratio of this Tm to that of diodrast is given by these observers as 2.35 in man and 1.0 in the dog; others (3) give the molar ratio as 2.2 in man and dog, but only as 0.8 in the rat.

Approximate values for glomerular filtration, plasma flow and tubular maximum useful in some clinical studies, may be calculated from data obtained after raising the blood PAH content to about 80 mg. per cent. Plotting the slope of decreasing plasma concentration, from timed blood samples, and measuring the amounts in consecutive 5 to 20 minute urine collections allows one to calculate values differing somewhat from those obtained by the classical method but avoids continuous infusion (4). On the other hand, quantitative constant infusion, after a priming dose, permits calculation of inulin clearance or PAH clearance from a single determination of plasma content (5). For when equilibrium is attained (30 minutes for PAH, 60 for inulin), the ratio is given by dividing the amount injected per minute by the plasma concentration. Water diuresis should be used in all precise determinations of ratios, as low urine volumes reduce, to variable degrees, the ratios of urea, inulin, creatinine, and some other solutes (6). The people of India are found to have ratios, for urea, only two-thirds those reported in North America (7).

In anesthetized rats (3), the mannitol ratio or GF is 0.0067 ml. per min. per gm., Tm for PAH is 0.0015 mg. per gm. of rat per minute. In this species water diuresis has little effect on inulin or diodrast ratios (8); in rabbits, water diuresis (9) or sodium sulfate diuresis (10) raises PF and GF; ether anesthesia (9) reduces the ratios; pentobarbital and mannitol or theophylline diureses have no effect, nor does posture (9). Glomerular filtration rate in rabbits is 15 ml. per min., plasma flow 50 ml. per min. (9). In dogs diodrast clearance is 191 ml. per min. per 100 gms. of renal tissue, inulin clearance is 64.0 ml. per min. (11). The ratios for sodium thio-sulfate, for dogs, are the same as the creatinine ratios, although

only 70 to 80 per cent of the injected material is recovered from the urine (12, 13). Because of low toxicity and ease of estimation this substance should be useful in estimating glomerular filtration, although the rate of outward diffusion through injured tubules can scarcely be as low as that of inulin.

*Effects of injury or disease on clearance estimates of glomerular filtration and plasma flow.*—This has been well reviewed by Laake (14), who summarized the literature in connection with an investigation of the changes in diodrast and inulin ratios in rabbits with uranium nephritis. Ekehorn, in a long and, at times, embittered polemic (15 to 22), criticizes the conclusions of Homer Smith and his co-workers. Noteworthy is his emphasis on the outward diffusion of solutes through damaged tubules, and on the inadequacy of the evidence that human and anthropoid kidneys excrete creatinine through the tubules. Ekehorn concludes that xylose and inulin are partly reabsorbed by the tubules of men and apes, and that the creatinine ratio gives the correct glomerular filtration.

The filtration fraction, FF, is obtained by dividing glomerular filtration by plasma flow. This is 0.3 in rabbits (9), 0.34 in dogs, and 0.26 is the creatinine FF in man. The human inulin FF is less than 0.2, the range 0.13 to 0.26. This remarkably low inulin FF, together with the comments of Ekehorn, cannot be ignored. It is possible that creatinine ratios more closely approximate true glomerular filtration in man, as in mammals in general, and in amphibia, including *Necturus* (25). Yet, in the damaged kidney, where all ratios diverge more and more widely from the true plasma flow and glomerular filtration, the inulin ratio probably gives the best indication of glomerular filtration rate, the Addis or urea ratio being most fallacious, and the creatinine or Rehberg test somewhat more reliable.

In patients with nephritis, there is no significant difference in the standard deviation of single determinations of inulin and creatinine ratios (23). In following the course of fifty cases, it was noted that when glomerular filtration is low, individual differences between inulin and creatinine ratios are small, but at higher levels, inulin ratios occasionally are higher than creatinine (? greater backward diffusion of the latter through the tubules). Difficulties with bad lots of inulin, causing errors up to 50 per cent have been encountered (23), but Josephson & Lindahl believe inulin gives closer approximations of glomerular filtration, in disease, than



does creatinine. In acute glomerulonephritis, urea ratios are often markedly lowered when diodrast ratios are normal, or only slightly lowered; in renal arteriosclerosis the reverse is often observed (24).

Patients with pyelonephritis, having normal or nearly normal urea ratios, show greatly diminished capacity to concentrate solutes in the urine (26). However, in studying the changes in human renal function with age, Shock (27) finds concentrating capacity least altered. Diodrast Tm and capacity to excrete alkali or acid begin to fall after the age of forty, urea and inulin ratios after fifty. Aging of the kidneys is as variable as that of the scalp; some subjects over seventy have renal function as good as that of others only twenty years old.

*Excretion of urate and other solutes with ratios much lower than glomerular filtration.*—In frogs and snakes direct measurements proved that the uric acid in glomerular filtrate was the same as in the plasma; the urate ratio equalled 30 per cent of the renal plasma flow (28). In man urate ratios give values one fifth those of urea, or less than 5 per cent of plasma flow. This is not greatly altered by water diuresis. In standard short tests in women the urate ratio is 14 ml. per min., and practically the same value is found in women who have recovered from eclampsia (29); corresponding urea ratio is 75 ml. per min. Using twenty-four hour urine collections to calculate the ratios, the following averages in ml. per min. are recorded: normals, urea 63 and urate 12.4; eclamptics, urea 42 and urate 6.4; recovered eclamptics, post partum, urea 55 and urate 10.4. In other words, with more concentrated urines, urate ratios fall more than urea ratios; both fall more in eclampsia and after it than is indicated by the ratios with high urine volumes in standard tests. A remarkable finding was that after raising blood glucose, in normal women, to more than 400 mg. per cent, the urea ratios rose only 25 per cent while those for urate rose 157 per cent (30). Protein binding of urate has not been proved to be the cause of the low ratios and it is difficult to see how this could be altered by raising the blood sugar. Reabsorption of the urate coming out of solution in the concentrated glomerular filtrate seems more probable; such reabsorption might be blocked or the urate held in solution by high glucose content of the filtrate.

Sulfathiazole, one of the most soluble of the sulfonamides, has an excretion ratio equal to or perhaps slightly above that of inulin (31), but its acetyl forms, which are less soluble, as well as

nearly all other sulfonamides, have ratios well below GF. The acetylated compounds usually have lower ratios than the free forms (31). Lundquist, who noted that *o*-iodohippuric acid excretion depressed the ratios of sulfathiazole and of sulfamethylthiodiazole, calculated the ratios for these substances using the plasma values corrected for the free (ultrafilterable) fraction (32). He concluded that sulfathiazole, with a ratio less than half that of inulin, and with only 15 per cent not protein-bound, must have been largely excreted by the tubules. Sulfamethylthiodiazole, with less than 8 per cent free, has a ratio 96 to 130 per cent of that of inulin; up to 95 per cent of this appeared to have been excreted by the tubules. One may question the use of *in vitro* determinations of diffusibility in the interpretation of such data. None of the sulfa ratios appear to exceed the creatinine ratio, but the depression of the ratios by hippuran or diodrast does indicate tubular excretion.

*Excretion rates of miscellaneous substances.*—Calculations of GF were used in studies of the excretion of many metabolites and other substances. Bicarbonate at plasma levels less than 20 mM per l. is completely reabsorbed from the filtrate. Above 30 mM per l. excretion occurs; the rate of reabsorption is independent of the plasma concentration, and averages 2.5 mM per 100 ml. of filtrate (33). Sodium *dl*-lactate is scarcely excreted as plasma levels below 1 mg. per ml. and higher levels can be maintained only by intravenous injection, not by feeding. Between 1 and 4 mg. per ml. excretion is proportional to GF (34). Lactate reabsorption is not depressed by high rates of glucose reabsorption, so that a single mechanism is not involved. Reabsorption and utilization of *l* exceeds that of *d*, the ratio  $d/l = 0.65$ . Maximal reabsorption, in dogs, is 80 mg. per min. Visscher reports that *dl*- $\beta$ -hydroxybutyrate is scarcely excreted at plasma levels under 8 mg. per cent; at that level in 30 kg. dogs, the sodium loss is 10 m.eq. per hour. Maximum reabsorption is 3 mg. per min.; at 35 mg. per cent the loss of sodium in urine is 400 m.eq. per hour (35).

Human excretion of amino acids appears to vary widely. Beyer *et al.* find that at "feasible" blood levels, *l*-tryptophane, *l*-leucine, *dl*-isoleucine, and *dl*-valine are not maximally reabsorbed and the ratios are very low (36). Eaton *et al.* (37) find that *dl*-valine and *dl*-isoleucine have the same reabsorption rates at low blood concentrations, with *l*-leucine somewhat more rapidly reabsorbed; at higher blood levels *dl*-valine is reabsorbed at the rate of 12 mg. per

min. per sq. m., the other two at 10 mg. per min. Feeding sucrose or glucose, 25 gm. after breakfast, lowers excretion of urea during the next three hours by as much as 150 mg., (38). Amino nitrogen, creatinine and arginine excretion fall less markedly; sulfate, indican, phenol, methionine, and histidine excretion are unaltered. Tryptophane fell from 12 mg. to 0 in one subject, less markedly in two others. Water diuresis abolishes the effects of carbohydrate feeding (38).

At normal blood levels, pantothenate gives very low ratios, above 0.5  $\mu$ g. per cc. the ratio rises to that for inulin (39). The cinchona alkaloids and bases, of which 25 per cent appear in the urine, give low ratios. When the blood levels for the bases are above 10 mg. per l., the ratios approach the inulin clearance, and the renal blood flow is increased, although the arterial pressure does not rise (40).

In less precise studies of excretion, 6 to 92 per cent of streptomycin is found in the urine after injection, averaging 50 per cent in four hours (41). When a 10 mg. dose of thiamine is given, more than 6 mg. is excreted in the next twenty-four hours; if 40 mg. is given, 90 per cent is excreted (42). Tubular participation in the excretion of estrogens is indicated by studies on the aglomerular toadfish (43).

*Some physiologic changes in excretion ratios.*—Changes in the volume flow of glomerular filtrate and in plasma flow are known to occur with effort and posture. In the erect posture (44, 45), inulin clearance falls 20 per cent, diodrast PF as much as 45 per cent, and the urine volume declines with a rise in specific gravity and inulin concentration. This occurs, and recovery occurs, within 15 min. of a change in posture. There is evidence that the posterior pituitary is involved in this postural oliguria (see p. 238).

After a 440 yard run, diodrast PF falls 18 to 54 per cent, inulin clearance usually falls. In half the subjects water diuresis occurring at the time of the run is inhibited. With longer runs, glomerular filtration always falls and diuresis is markedly reduced (46). Pressure breathing, which has been used at very high altitudes, decreases cardiac output and renal functions; intermittent pressure is less deleterious than continuous pressure (47). Glomerular filtration and plasma flow only occasionally rise when rabbits are made hypertensive by carotid denervation; if the kidneys have already been denervated, all show rise in glomerular filtration and

the majority a rise in plasma flow (48). In a few oliguric cases of nephritis, one due to arsenic poisoning, rises in glomerular filtration followed intravenous novocaine (49). In one case the creatinine clearance rose from 104 to 255 ml. per min. after 20 ml. of 1 per cent solution of scurocaine (50).

#### EFFECTS OF SHOCK AND OF CONSTRICTION OF RENAL ARTERY ON RENAL BLOOD FLOW AND OXIDATION RATES

In cats, the fall in renal blood flow (diodrast PF) is small compared to the fall in blood pressure following bleeding. The difference is striking in the early stages, but even in the terminal stages, when pressure and renal flow are very low, the flow is better maintained than it is in cats when shock is produced by tissue damage due to tourniquets on the lower limbs (51). In tourniquet shock the percentage fall in renal blood flow always is greater than that in arterial pressure. In the shock due to injection of adenosine-triphosphate (ATP), the percentage fall in renal flow is much less than that in arterial pressure (52). In some cases, renal flow increases as pressure falls. This seems to disprove the claim that ATP, or its magnesium salt, is the substance causing tourniquet shock. As ATP caused fever (53), and as fever-producing protein injections cause a rise in renal blood flow, the rise in flow from ATP is not unique.

Dogs show a very different response to hemorrhage. Phillips and his co-workers (54) observed an initial fall in arterial pressure and in plasma flow, with prompt return to normal; then a decline in plasma flow with glomerular filtration and blood pressure well maintained, and terminally plasma flow and glomerular filtration fall to zero, before mean pressure has fallen below 80 mm. Hg. Selkurt (55, 56) lowered the arterial pressure of dogs to 60 mm. Hg. by bleeding and held it there for 40 minutes. The immediate fall in blood flow through the kidney (direct measurement) was 59 per cent of the control rate but by the end of the 40 minutes, the fall was 89 per cent. By reinjecting blood, arterial pressure was then brought to normal; the renal blood flow rose to only 80 per cent of the control rate. PAH ratios indicated a plasma flow much below that measured directly, and it was apparent that PAH excretion was reduced, creatinine reabsorption increased as a result of tubular injury. The outstanding fact is that cats have renal vasodilata-

tion, dogs renal vasoconstriction as a result of severe hemorrhage; both have vasoconstriction in tourniquet shock.

Dogs in shock, with low renal blood flow, exhibit the same striking fall in renal oxygen use which was noted with clamps reducing the renal blood flow (57). Dole and co-workers (58) report that even in early stages of shock, renal oxygen uptake falls proportionately to the fall in blood flow. The normal kidney has a low oxygen extraction ratio or arterio-venous difference. This remains low as PF falls to very low levels and renal oxygen uptake becomes very low in shocked dogs. In other organs, oxygen extraction rises, and oxygen uptake per minute is little altered. When the arterial oxygen tension was reduced, by having dogs breathe 10 per cent oxygen mixtures, plasma flow rose and oxygen uptake per minute did not fall. In the shocked dogs, PAH extraction ratios remained high at lowest levels of renal blood flow, indicating relatively slight tubular damage until blood flow almost ceased.

Temporary complete occlusion of a renal artery in dogs for three minutes causes an 8 per cent fall in the PAH ratio after releasing the artery; a ten minute occlusion, a 41 per cent decrease; a twenty minute occlusion, an 81 per cent decrease; twenty minute occlusion during a mannitol infusion causes only 34 per cent decrease in PAH ratio (59). Creatinine GF and urine flow also were markedly reduced after the 20 minute occlusion; recovery was slow—120 minutes after a ten-minute occlusion. This prolonged renal vasoconstriction contrasts with the immediate intense reactive hyperemia in other tissues after cutting off arterial flow—for example, a twentyfold increase in blood flow in the arm after ten minutes of ischemia.

Probably related to the inhibition of oxidation and absence of reactive hyperemia, when renal blood flow is reduced, is the kidneys' resistance to prolonged anoxia. One third of a group of rabbits survived two hours of total renal ischemia (60); there was no rise in arterial pressure. All of a group of dogs survived one or two hours renal anoxia, and 50 per cent survived four hours compression of the artery supplying the remaining kidney after unilateral nephrectomy (61). Uremia and marked but transient tubular changes follow such insults, but in surviving animals the kidney appears normal and functions normally within a few weeks. It must be emphasized that while simple anoxia with normal blood flow has no such effect, reduced renal blood flow causes a very

marked inhibition of oxidative systems in the kidney. Either because vasoconstrictors are evoked, or substances sensitizing the arterioles to epinephrine (62), or because oxidation itself is reduced, reactive hyperemia is absent and reduced blood flow persists after normal pressure in the main renal artery has been restored. This reaction pattern, coupled with emergency vasoconstriction of the kidney during stress or after injury to the body, permits a shunt of nearly one fourth the cardiac output from the kidney to other tissues without harm to the kidney unless the emergency lasts many hours.

*Relation of renal oxidative systems to renal vasoconstrictors.*—The enzyme renin, obtained from normal kidneys, acts upon a substrate in the blood plasma, hypertensinogen, to form a pressor polypeptide angiotonin or hypertensin, to which the renal vascular bed is itself extremely sensitive. Other enzyme systems producing vasoconstrictors may occur in the kidney (63), and gastric juice, acting on plasma at pH 7, also forms hypertensin. Mammalian plasma inactivates hypertensin and the hypertensinase titers of plasmas from many sources closely parallel the aminopeptidase titers (64). In some species, following renal ischemia or renal arterial constriction, renin can be demonstrated in blood from the renal vein, or even from other veins, but it is not demonstrable in most cases of hypertension, whether of renal origin or not. Renin, then, may simply be a proteolytic enzyme without specific function or a part of the mechanism for controlling renal blood flow and even, in emergency, for raising systemic pressure. The kidney is rich in such catheptic enzymes. Recent estimates (65) give the following figures: pepsinase, 22 units in cortex, 4.7 in medulla; aminopeptidase, 16 in cortex, 3 in medulla; carboxypeptidase, 12 in cortex, 2.8 in medulla; and trypsinase, 11 in cortex, 3.9 in medulla. The values correspond with the difference in rates of autolysis observed in cortex and medulla; in the cortex autolysis is far more rapid in the proximal than in the distal convoluted tubules. It is also in the proximal convolutions that proteins, reabsorbed from the glomerular filtrate under abnormal conditions, appear to undergo lysis (66).

Substances which reduce tissue oxidation also occur in the kidney and are increased by renal ischemia or by constriction of the renal artery. Reduced oxidative activity in slices from kidneys of animals with renal hypertension has been noted by various ob-

servers (67). Cytochrome C, cytochrome oxidase, and succinic dehydrogenase are found by Raska (68) to be reduced 50 per cent or more from control values in the slices and homogenized suspensions from dog's kidneys after arterial constriction, and elevated in the contralateral kidney. After unilateral nephrectomy an increase occurs in these substances and also in flavin-adenine dinucleotide, coenzymes I and II, and in amine oxidase. Such increases precede hypertrophy. Suspensions from kidneys causing chronic hypertension, and renin prepared from normal kidneys, inhibit these oxidative systems, but preparations from normal kidneys are much less active inhibitors than those from kidneys with chronic renal arterial constriction. The claim has been made (69, 70) that the kidney opposite one with a clamp on the artery contains more material which destroys hypertensin, or hastens its oxidative destruction by tissue slices, and also contains more of a dialyzable substance which lowers blood pressure in animals with renal hypertension than does the normal kidney.

While research concentrates on relief of hypertension, the biologist is concerned less with the production of pressor substances which have not been proved specific in origin or in site of action, than with the striking inhibition of oxidation evoked by reducing the arterial inflow or pulse pressure, for this seems to be a phenomenon peculiar to the kidney and unique in physiology.

*Other peculiarities of renal oxidation.*—Renal tissue of rats and rabbits cannot decarboxylate tyrosine, but can decarboxylate *l* or *dl*-dihydroxyphenylalanine (Dopa) aerobically or anaerobically (71). Slices of guinea pig kidney show a 50 per cent increase in oxygen uptake when Dopa is added, but this does not occur if the slices are obtained from pigs in a scorbutic state (72). Feeding Dopa to the pigs increases their need for ascorbic acid. Slices of kidney have higher rates of amino nitrogen and lower rates of ammonia production under anaerobic than under aerobic conditions; this is the reverse of what occurs in liver slices (73). With the use of carbon isotope it is found that neither citric acid, nor *cis*-aconitic acid are intermediates in renal oxidation of acetate (74).

#### ARTERIAL HYPERTENSION AND THE KIDNEY

No new light has been shed on the relative incidence in man of hypertension of renal origin, nor on the mechanism of chronic renal hypertension. In dogs or rabbits, made hypertensive by de-



pressor nerve section, renal vasoconstriction or participation of the kidneys in the elevation of blood pressure is not demonstrable (76, 48). The splanchnic or the renal nerves of dogs can be stimulated with sinusoidal currents, for many hours and over many days (77, 78). At 2 cycles per second there is an immediate rise in arterial pressure when the splanchnics are stimulated, but only an occasional delayed rise in pressure on bilateral renal nerve stimulation. With the latter there is an initial fall in plasma flow and glomerular filtration, but a return toward normal in two to four days. Stimulation can be maintained for twenty-two hours a day, and in one dog a sustained fall of 30 per cent in plasma flow, a rise in filtration fraction, and a rise in blood pressure to 200/135 mm. Hg. occurred. After twenty-seven days stimulation was discontinued and the changes rapidly disappeared. It has not been determined whether the kidneys play a part in the hypertension of rats exposed to constant or repeated noise (79). The renal element in hypertension has been summarized briefly by Nicoll (80). There is additional evidence that renin and a substrate formed in the liver are responsible for the acute rise in blood pressure of dogs after narrowing or occluding the renal artery (81 to 86). There is no proof that a peripherally acting vasoconstrictor of renal origin plays a part in chronic renal hypertension. Von Euler & Sjostrand (87, 88) could demonstrate no more pressor substance in extracts of blood and urine of hypertensives than in controls.

Wakerlin and co-workers report further observations on renin content of kidneys and the effect of extracts rich in renin in the prophylaxis and treatment of experimental renal hypertension (89 to 92). Stevens could demonstrate no value in such therapy in hypertensive patients (93).

The juxtaglomerular tissue has again been reported to have a rich nerve supply (97), and to undergo hyperplasia after shocking injuries to the body (98, 99), or even after trauma to the kidney itself (100). A relation of this tissue to renal vasoconstriction and inhibition of oxidation has not been proved.

Kempner feels that the excellent remissions in more than half the hypertensives he has treated with a diet of rice, fruit and sugar are due to reducing the metabolic load on the kidney (101, 102). The diet is lower in sodium than any previously used, and Grollman and co-workers (103) confirm the old reports of similar remissions on salt-poor diets not so low in protein. In rats with renal

hypertension, a drop of pressure to normal occurs within six days, on a diet nearly free of sodium (104). Such a diet, like the fish oil extracts which lower the pressure (105), prolongs the animals' survival time. Perera *et al.* find that hypertensive patients who develop Addison's disease (adrenal insufficiency) show normal blood pressure, but on desoxycorticosterone therapy return to hypertensive levels (106, 107). It is clear that a negative sodium balance soon abolishes hypertension, but what part the kidney plays in this, and what factors aside from sodium are important, remain unsettled. The value of rice as a source of protein, when low protein intake is desirable, is proved by Sure's observation that rice protein is two and one-half times more efficient than wheat protein in maintaining the growth of rats (108). Per calorie, white wheat flour contains more than four times as much sodium as polished rice.

In a six-year-old child with severe constant hypertension, removal of a small scarred kidney was followed, within an hour, by a fall to normal levels, maintained during eighteen months' observation (109). Two similar cases in older patients had as prompt falls, observed to last nine and twenty-four months (110). Unilateral renal lesions causing hypertension are rare, but cannot be ignored.

The relationship of the kidney to hypertension is also considered in the chapter on peripheral circulation in this volume.

#### THE EXCRETION OF WATER, OF SODIUM, AND OF POTASSIUM

The proximal tubules of the frog's kidney can accumulate intracellular potassium, against a gradient to the surrounding fluid, until the potassium reaches three times its normal intracellular concentration (111). With this there is no increase in cell volume. When potassium is substituted, in increasing amounts, for the sodium of an isotonic solution, the volume of the kidney finally is more than doubled, and the water content of the cells of the proximal tubule is four times greater than normal. These cells are impermeable to sodium, while those of the distal convolution are permeable to sodium and accumulate no potassium. The cells of the distal part of the tubule can extrude sodium and the osmotically associated water until the kidney loses 15 per cent of its fresh weight. The authors conclude that reabsorption of water and sodium occurs only in the distal part of the frog's renal tubules,

The renal aspects of the regulation of serum electrolyte are discussed in Broch's monograph (112) in which are noted the paradoxical conditions of edema with hypochloremia, and dehydration with normal chloride levels. Wolf describes a formula for calculating the "retention threshold" for sodium or chloride from the data obtained during the steady state of excretion in men given intravenously 7 ml. per min. of saline, of varying concentrations, for seven hours (113). In dehydrated subjects, the osmotic pressure of the urine falls during diuresis evoked by administering hypertonic sodium chloride solution; lowering the salt intake leads to a fall in minute volume of urine and a rise in urea and potassium concentrations (114). But when urine volume is above 3 ml. per min., giving urea in addition to salt depresses the salt content of the urine, with little or no rise in osmotic pressure (115). This is interpreted as meaning that at each rate of urine flow, there is a limiting osmotic pressure, which falls with rise in urine volume, and that tubular reabsorption of water is not limited by a maximal chloride level.

An intense diuresis, nearly nine times the control rate, evoked by intravenous reinfusion of urine in dogs, is contrasted with the mild diuresis caused by intravenous saline (116). Caffeine diuresis in rabbits increases excretion of ammonia and reduces that of urea. Ammonium chloride alone increases excretion of both substances, but if given with caffeine the ammonia excretion rises and urea excretion falls markedly (117). Equimolar doses of mercupurin and of mercurhydrin are equally effective diuretics in man (118). Williams (119) reports seven males in five generations of a single family with diabetes insipidus refractory to pituitrin. Fluid intakes were as high as 18 l. per day and excretion ratios suggest other tubular defects less severe than that in water reabsorption. The defect was transmitted through the females, who showed no instance of the disorder.

Glycosuria, up to 1,000 milliosmols per day, was induced in diabetics by withdrawing insulin or administering glucose. Urine volumes rose two-to-five fold, but chloride excretion was not significantly increased (120). Sodium excretion may be increased forty fold during high rates of excretion of  $\beta$ -hydroxybutyrate (34). At simulated altitudes of 4.5 to 6.5 km. excretion of base is two or more times that of controls at the same hour and on the same diet (121, 122). During one hour at low pressure, sodium con-

centration in the urine rises 50 to 400 per cent, average 200 per cent; potassium only 0 to 225 per cent, average 71 per cent. During the next hour, with recovery at normal pressures, excretion of base is still high, that of potassium higher than in the previous hour and above the sodium concentrations (123).

*Sodium and water retention; the role of hormones.*—In men on low sodium intake, the twenty-four-hour output in the urine falls to 40 mg. (124). This is true even in diabetes insipidus with a urine volume of 8 l. if the sodium intake is 250 mg. per day. A severe chronic nephritic, on the other hand, may lose 750 mg. on a 300 mg. intake. Patients with relapsing fever, though taking 2 l. of milk (1 gm. of sodium) daily also excrete less than 40 mg. of sodium per day during the paroxysms of fever. There is weight loss, increase in urea excretion, rise in spinal fluid sodium chloride content, and little or no edema. This phenomenon occurs but is less marked in pneumonia, typhus, etc. (125). It is noteworthy in this connection, that with the onset of most fevers, renal blood flow and glomerular filtrate volume increase; in spite of this sodium loss is greatly diminished.

Merrill (126) has accepted Stead's thesis that sodium retention in heart failure is due to reduction in renal blood flow, in glomerular filtrate, and therefore in sodium "presented to the tubules for re-absorption." By using the inulin ratio he calculates the average normal subject has 18.1 m.eq. of sodium present in one minute's glomerular filtrate, of which only 0.22 m.eq. are excreted. This totals 7 gm. per day. In five cardiacs, 6 to 12 m.eq. were present in the filtrate, and when not under the influence of mercurial diuretics (which raised the minute excretion to as much as 0.15 m.eq.), the sodium excretion was 0.0013 to 0.02 m.eq. per min. These excretion rates, 42 to 60 mg. per day, are no lower than are excreted by normal men, or cases of diabetes insipidus, on low sodium intakes. The patient with the lowest excretion happens to be the one with the largest amount of sodium "presented to the tubules." Merrill ignores the possibility that sodium retention in heart failure is an expression of a homeostatic mechanism evoked whenever cardiac output, per minute, fails to meet the demands of the body. The possibility of hormonal influences is also ignored.

Brun (44, 45, 127, 128), in his discussion of postural oliguria, regards the salt and water retention as homeostatic mechanisms involving the posterior pituitary. The involvement of the adrenal

cortex also may be suggested. In a subject who faints on the tilt-table, oliguria persists as long as ninety minutes, and a transfusion from the subject inhibits the diuresis of a well-hydrated recipient (128). In cases of diabetes insipidus, postsyncopal oliguria is mild and brief. The absolute rate of chloride excretion falls during postural and postsyncopal oliguria. As is well known, the rate of chloride excretion rises during the oliguria evoked by pituitrin (129, 130), although not in that evoked by extracts of urine from dehydrated subjects (130). Brun, recalling Asmussen's observation that men always have latent circulatory failure when erect (131), believes that the antidiuretic mechanism is set off by "latent failure."

Exercise (132) inhibits water diuresis and alcohol diuresis. Emotional stress, in dogs, inhibits water diuresis, but this inhibition is almost abolished by section of the supraoptico-hypophyseal tract (133). Heat abolishes water diuresis, even if the loss of sweat is made good every twenty minutes (134). During acclimatization to heat (and also in heart failure) the sodium concentration in the sweat decreases almost one half. Desoxycorticosterone causes the same change to occur in a temperate environment but if given before or during acclimatization to heat, and then discontinued, acclimatization is lost, the sodium content rises, and reacclimatization must occur (135). It seems probable that the sodium retention in many of these conditions is due in part to adrenal cortical hormone, liberated in response to nervous stimulation of the anterior hypophysis or of the adrenals.

Malmejac and co-workers (136 to 140) studied the effects of low barometric pressure on renal function of dogs, with intact or transplanted (denervated) kidneys, after adrenalectomy, and in isolated perfused kidneys. In perfused organs renal blood flow was reduced only when gas emboli appeared. At pressures equivalent to 9 km. altitude, renal blood flow and urine formation ceased, but complete recovery occurred within four minutes of recompression. With kidneys in the animal, emboli were suspected but not proved. The adrenals and other factors seemed to play a part in causing oliguria; chloride excretion was reduced with the oliguria.

The reviewer concludes that severe arterial anoxia does not lower renal excretion of sodium, but increases it (121, 122); sodium retention in many other conditions is a result of the mechanism to increase blood volume, tissue fluid volume, and thus, cardiac out-

put, when it is below the needs of the body. Under natural conditions in animals or man, this mechanism is usually evoked by hemorrhage, trauma, food poisoning, heat, and violent effort.

Ralli and co-workers (141) find a large amount of antidiuretic substance in the urine of cases of cirrhosis of the liver with ascites. They suggest that the failure of a damaged liver to catabolize such a hormone may contribute to the anasarca and ascites of advanced liver disease. These cases had lower urine volumes and higher specific gravities than the controls, as would be expected, since both had the same fluid and salt intake and the cirrhotics were retaining considerable fluid. This in itself might stimulate liberation of antidiuretic hormone, which has been found regularly in the urine during dehydration.

*The pituitary: water and salt retention.*—After injury or destruction of the anterior lobe of the hypophysis, water diuresis is diminished (142) and excretion ratios fall. Saline becomes a more effective diuretic than water. No repair of renal defect is noted after treatment with thyroid, suprarenal cortical extract, or desoxycorticosterone. Water diuresis increases after removal of the posterior lobe, or after section of the supraoptic tract, which causes the most prolonged diabetes insipidus.

The adrenocorticotrophic hormone of the pituitary, within an hour after its injection into animals, causes effects similar to those produced by material which can be obtained from normal urine. The excretion of this material is diminished in Addison's disease and panhypophyseal deficiency, but increased in the urine in Cushing's syndrome, after burns, operations, or severe effort (143, 144). This material may be 17-hydroxy-11-dehydrocorticosterone, for its biological properties are similar. It increases glycogen deposition in the liver, protects in the cold test, prolongs life, prevents water or potassium intoxication in adrenalectomized mice, and also increases sodium excretion (145). As sodium is retained in Cushing's syndrome, this substance must be produced less abundantly than desoxycorticosterone which has an opposite effect on sodium excretion and which apparently also is evoked by hypophyseal and hypothalamic influences (146).

It had been noted by Ham & Landis (130) that antidiuretic extracts of urine had none of the chloruretic effects of pituitrin, and that dialysis removed this activity from postpituitary extracts or powders. Dicker & Heller (147) now report that pitressin

has no chloruretic effect, but reduces renal plasma flow, increases the filtration fraction and even glomerular filtration, and reduces diodrast Tm from 0.13 to 0.097 mg. per min. Pitocin, on the other hand, increases plasma flow, greatly increases glomerular filtration has no effect on Tm but reduces reabsorption of chloride by the tubules. Whole pituitrin lowers Tm and chloride reabsorption, reduces plasma flow and slightly raises glomerular filtration. These observations on rats must be extended to other species. The effect of pitocin on sodium excretion and its relation to the adrenal cortical substances are of immediate interest. It would appear that the antidiuretic action of urine extracts and oliguria without chloruresis may be due to a pure pitressin effect. A simultaneous decrease in pitocin liberation could explain chloride retention, as could a decrease in liberation of 17-hydroxy-11-dehydrocorticosterone and an increase in liberation of desoxycorticosterone.

#### RENAL MORPHOLOGY

Oliver (66) reviews the contributions to knowledge of the structure, growth, and abnormalities of the nephron made possible by microdissection. In work hypertrophy of the rat's kidney, produced by subtotal nephrectomy and a high protein diet, the proximal convoluted tubule increases tenfold in volume; the volume of the nephron and its vascular and supporting structures increase 2.4 times. In normal growth, there is a linear relation of the logarithms of the volumes of the proximal tubule and the nephron. The volume of the glomerulus has a similar linear relation, but with slower growth, to seventy days; thereafter the slope is again constant but growth is much more rapid. In male rats, the ratio of the logarithms of kidney and of body weight is linear during normal growth, but there are inflections at five and twenty-seven days (148). Up to five days  $k=1.44$ , from five to twenty-seven days, 1.02, and from twenty-seven to two hundred and eighty days, 0.81.

Glycogen deposition, in diabetes, occurs in men chiefly in the terminal part of the proximal convolutions; in rats, cats and dogs, it is found only in the ascending limb of the loop of Henle. Lipid is demonstrable in the proximal part of the proximal convolutions of the healthy cat, in the medullary portions of the tubules of the dog. Lipid is not normally seen in human nephrons, but in lipoid nephrosis it is present, in spotty distribution, in the proxi-



mal convolutions (66). Human kidneys show fatty change in the medulla more often than in the cortex (149); the anisotropic lipids are seen only in nephritis and essential hypertension. The fat usually seen appears to be neutral fat typical of that in the depots throughout the body.

Calcium deposition, in rats on high phosphate diet, occurs in the terminal part of the proximal convolution and the mid-portion of the ascending limb (66). Protein and gelatin, in animals and man, accumulates in the proximal convolutions, and is most abundant near the glomerulus or in the mid-portion (66). Here it forms vacuoles and displaces the mitochondria.

Cast formation usually is limited to the distal convolutions and collecting tubules; casts show the metachromatic staining characteristic of chondroitin or mucosin sulfate conjugated with protein. Normal urine contains a substance having the characteristics of such a sulfate. Bence-Jones protein forms casts even in the proximal convolutions. While albumin is precipitated by chondroitin sulfuric acid, or by the urinary congener, only at pH 4.2 or below, Bence-Jones protein is strongly precipitated by pH levels up to 6.4 and to some degree at much higher levels (66).

*Renal pathology.*—Bradley's review (150) of renal insufficiency emphasizes the extra-renal factors. Myoglobin liberation and renal ischemia from shock play important parts in "crush syndrome" (151). Bywaters (152) reports tubular lesions in hydronephrosis similar to those in "crush syndrome," and Bergstrand describes similar lesions in twelve cases of sulfathiazole damage to the kidney (153).

After unilateral nephrectomy in rats, there is no rise in mitotic figures on the first, or on the third and following days (154), but on the second day three times the control number of mitoses are present in the cortex, twice as many in the outer, and four times as many in the inner part of the medulla. This resembles the phenomena in regenerating liver in the same species where the number of cells does not increase on the day following subtotal hepatectomy, increases 58 per cent on the second day, 25 per cent on the third and less thereafter (155). It is possible that mitosis is not only more frequent but is less rapid on the second day of intense compensatory hypertrophy, so that the number of cells found in mitosis is maximal then.

In hypophysectomized rats there may be no renal hypertrophy

after unilateral nephrectomy (156). Thyroidectomy retards renal growth, but does not prevent compensatory hypertrophy (157). While inanition diminishes the weight of kidneys, deficiency of B complex causes a 50 per cent increase in their weight (158).

BAL, British anti-Lewisite, (2-3 dimercaptopropanol) protects against mercurial necrosis and lowers death rate in humans poisoned by bichloride (159). In dogs, phloridzin protects a third of the animals from the renal damage following 4 mg. per kg. of mercury bichloride (160). The renal ratio for mercury is one third to one half that of inulin. The inulin ratio is not altered by phloridzin, that for mercury is halved by the glucoside. The tubular damage due to mercury, dichromate, and especially to uranyl causes glycosuria (161), without relation to the blood glucose, blood urea, or the levels of alkaline phosphatase in blood or renal cortex. Wachstein (162, 163) found changes in phosphatase and in lipase were parallel in injury to tubules caused by mercury, uranium, choline-deficiency, and ureteral ligation. Lipase is normally present in the proximal convolutions of rat, rabbit, dog, mouse, hamster and guinea pig, but not in man. It is present in necrotic tubules of animals after acute injury, but disappears in regenerating or atrophic tubules. In the acute stage of choline deficiency injury, it also is depleted.

In rats the nephrotoxic and diabetogenic doses of alloxan are the same (164); in dogs the islets are more susceptible (165). In rabbits alloxan injures the tubules in the same way as mercury (166). Early deaths after a single dose of 200 mg. per kg. intravenously are due to hepatic necrosis; but uremia killed eight of fourteen animals dying after the fourth day. The hepatic and renal necrosis caused by pyridine can be prevented by methionine (167) and is not produced by equivalent doses of methyl pyridinium, the substance presumably formed by methylation of pyridine in the body. The injury therefore may be due to demethylation. In human disease, where hepatic and renal injury or necrosis are found, the severity is parallel in the two types of tissue in each case, regardless of the severity of jaundice at the time of death (168). It is concluded that the renal lesions in cirrhosis and hepatitis are due to the same toxic or deficient states. Dogs, on a diet of rice, sugar and lard, with yeast, percomorph oil and salt mixtures but no protein, develop severe renal tubular damage in three to four weeks, when plasmapheresis (10 cm. per kg. twice

weekly) is performed. Blood phosphate and NPN rise and there is proteinuria, fall in serum albumin but no rise in cholesterol (169). Alkaline phosphatase is decreased, acid phosphatase increased in kidneys hypertrophied by implantation of androgenic steroid (170). Potassium cyanide 0.01 *M*, almost completely inhibits renal alkaline phosphatase, but the effect is reversible if the cyanide is washed off the slices (171). In the intestine, however, 0.001 *M* KCN completely inhibits phosphatase, and the effect is irreversible.

Chloroform, given orally to mice, causes a necrosis of the proximal and distal convolutions in male mice, or in males castrated and treated with testosterone, but not in females or in castrated males (172, 173). The normal males, and the castrated but treated males, have over 64 per cent of the Bowman's capsules lined with cuboidal cells, while female mice have squamous cells lining all but 31 per cent, and castrate males all but 20 per cent of the capsules. Neither the capsular lining nor the cells of the adjacent part of the tubules show necrosis from chloroform, so the relation of the anatomical and the pharmacologic differences is not explained.

In strain *A* mice, but not in other strains or rats or rabbits, urethane given once a week for ten to thirteen weeks leads to extreme hyalinization of the glomeruli (174). Dogs permanently diabetic after treatment with anterior pituitary substance, were treated with 30 U-insulin daily for five years (175). They had high blood cholesterol levels for four years at least, but the blood pressure and urinary protein levels are not reported. All developed glomerulosclerosis similar to the Kimmelstiel-Wilson lesions which develop in many human diabetics after years of successful management.

After unilateral nephrectomy rats given sodium chloride and anterior pituitary injections develop large adrenals and nephrosclerosis (176). Adrenal cortical extract does not cause or prevent such renal lesions, but adrenalectomy prevents their occurrence. Chicks, given 3 per cent sodium chloride in the diet, have a high water intake (up to 50 per cent of body weight per day), become edematous and show glomerular hypertrophy (177). Selye (178) observes that desoxycorticosterone (DCA) causes nephrosclerosis of rats, and this is augmented by unilateral nephrectomy and salt feeding or by thyroxine. Testosterone causes renal hypertrophy

without nephrosclerosis, while anterior pituitary injections cause both sclerosis and hypertrophy. In rats with nephritis caused by injecting serum containing antibody for renal tissue, DCA causes severe hypertension, glomerular hypertrophy and damage, and tubular injury (179). DCA has no effect on normal rats, and adrenal cortical extract has no effect on the kidneys or the blood pressure of rats with serum nephritis.

#### PROTEINURIA

Oliver (66) and others, accept the passage through the glomeruli of protein as a normal or "quasi-normal" phenomenon. Tubular reabsorption may be demonstrated in various ways in rats (66, 180). Smetana, using dyed serum albumin, confirms this as normal in urodiles, mice, and rats, but found no dye in the tubule cells of normal rabbits, guinea-pigs, or dogs (181).

When gelatin is given intravenously to men, in 50 gm. doses, 30 to 50 per cent is excreted in the urine, the first day, together with 10 to 15 gms. of serum protein (182). The proteinuria may be due either to glomerular injury, of which there is no other evidence, or to the gelatin blocking normal reabsorption of albumin from the glomerular filtrate. If the latter contained 10 mg. per cent of protein (less than in spinal fluid, tears, or any other body fluid) the total amount in one day's filtrate would be 18 gms.

Normal men have less than 1 mg. per cent of protein in the urine (183), and rarely have more than this after exercise (40 knee bends). However, in patients with heart failure, the urine often contains over 2 mg. per cent of protein at rest. In such cases, and also in nephritics, the mild exercise increases proteinuria. In cardiac patients with less than 1 mg. per cent proteinuria at rest, the concentration rose above 2 mg. per cent after the exercises. Whether this is due largely to greater concentration, or to changes in rate of leakage, or of reabsorption of protein has not been studied.

Faber, noting a rising ratio of serum cholinesterase to serum albumin in cases of chronic proteinuria with low blood albumin levels, concludes this is due to lower rate of urinary loss of esterase, with both substances being formed by the liver at constant ratio (184). There is reason to doubt that albumin formation is quantitatively, or even qualitatively, normal in such cases. Confirming older studies (185), Faber finds that the serum albumin in

these patients has only half the normal cystine content (186), while the serum globulins and the albumin in the urine have a normal content of this amino acid. The serum protein contains a fraction, not normally demonstrable, which precipitates with albumin but unlike the latter, remains insoluble in salt-free water. This fraction is poor in cystine (186).

Chronic albuminuria, with daily protein loss of from 5 to 15 gm. in the urine, usually leads to a fall in serum protein, especially in the albumin fraction and a rise in blood lipids, including cholesterol. No such changes in the blood occur with repeated removal of much larger amounts of plasma protein per day in donors of blood to whom red cells are returned (187), nor in cases of Benec-Jones proteinuria where damage to the tubules seems to be equally severe, although of a different character. In the latter the tubules are choked by precipitated protein, even in the proximal convolutions; in nephrosis these tubules contain no precipitate but the cells are filled with protein and lipid. In animals with proteinuria, the accumulation in these cells of dyes absorbed by serum albumin suggests that the rate of protein reabsorption may exceed the rate of protein loss in the urine. In the kidney reabsorbed protein may not only be split to amino-acids, but may be largely deaminized. Such a process might lead to the perverted metabolism of albumin and lipids noted in patients with proteinuria.

In nephrectomized rats, 25 to 30 per cent less urea is formed per day than in the intact animals (188). Some of the urea of the intact animal may come from ammonia produced in the kidney from materials in the blood, but part of it may come from proteins reabsorbed from the glomerular filtrate. In that event animals with severe proteinuria from glomerular disease might show a much greater fall in urea formation after nephrectomy.

When patients with proteinuria receive 25 to 50 gm. of human serum albumin daily by vein (189), there is an increase in daily protein excretion. The retention of protein varies from 60 per cent after a 50 gm. total dose, down to 17 per cent, after a 1500 gm. total dose. In some instances (189) (Figs. 6, 8 and 9) there apparently is a slight increase in excretion of nonprotein nitrogen, in others a fall. Occasionally (189) (Fig. 4) the nonprotein nitrogen excretion is almost doubled and protein loss tripled. The rise in protein excretion varies from 20 to 200 per cent of the control

rate, and appears not to be directly related to rise in serum albumin or in volume of glomerular filtrate, although no precise data on the latter are given.

*Relation of the kidneys to metabolism of fats and carbohydrates.—*

In dogs, transient rise in cholesterol, lecithin and total lipids of the plasma follows many types of renal injury, including unilateral and bilateral nephrectomy (190, 191). The kidneys help to maintain survival after evisceration, and fasted rats with the kidneys intact survive twice as long as those which have not been fasted. After nephrectomy, this difference disappears (192). Gluconeogenesis in the kidney appears to be more rapid in fasted than in fed animals. Rats fed a high-fat diet also survive evisceration and maintain blood sugar better than those on stock diet, if the kidneys have not been removed (193). This seems to be due not to gluconeogenesis but to a glucose-sparing action. In rats on stock diets, there is no rise in the fatty acid content of the kidney after evisceration; in fat-fed rats the fatty acid content rises, and the investigators suggest that lipids may be supplied by the kidney and utilized by other tissues.

METHODS OF SUPPLEMENTING OR REPLACING THE EXCRETORY  
FUNCTION OF THE KIDNEY

Two methods for performing the excretory work of the kidney have been applied to patients with acute renal insufficiency, and observations on the use of such methods in animal experiment have been reported.

The purely mechanical method of vividialysis is made possible by complete heparinization. With cannulae in veins and artery (or from vein to vein by use of a pump) blood may circulate through semipermeable tubes bathed in a fluid with proper electrolyte and glucose content. With fine collodion tubing which permits use of a small volume of blood and provides 24,000 sq. cm. of surface for dialysis, Kolff & Berk (194, 195) have removed up to 250 gm. of urea in fourteen hours from a patient.

Passing the dialysis fluid through a loop of gut, or over a serous surface, also permits removal of metabolites. The former method is ineffective, but by injecting the fluid at the rate of 40 ml. per min. into one side of the dog's peritoneal cavity and collecting it from the other, urea can be removed at 40 to 75 per cent of the normal renal rate (196). Peritoneal irritation, and peritonitis in

spite of antibiotics, are predictable complications of this method and have been observed in clinical application, although the patient's life was saved. The blood urea fell from 125 mg. per cent to 40 in four days of treatment (197), although renal function was nil.

Both methods are undoubtedly susceptible to greater refinement, and some modification may even be applicable in chronic renal failure. The study of renal function, and particularly of the hormonal and metabolic functions of the kidney, remains incomplete as long as the excretory function cannot be satisfactorily replaced after ablation. The tidal irrigation of an isolated lobe of the lung with a solution of suitable chemical and colloidal composition offers the most physiological method, for the lung, an exchange organ, is not injured by contact with gas, dusts, and bland fluids, and offers an ideal surface for dialysis.



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DEPARTMENT OF MEDICINE  
LONG ISLAND COLLEGE OF MEDICINE  
BROOKLYN, NEW YORK

## PERIPHERAL CIRCULATION

BY CARL J. WIGGERS<sup>1</sup>

*Department of Physiology, Western Reserve University  
Medical School, Cleveland, Ohio*

This review is intended to interpret the essential contributions published between August 1, 1944 and August 1, 1946, except those covered in previous reviews. Older references are introduced only when demanded for an integrative interpretation. Occasionally, attention is directed to pertinent reports dealing with peripheral circulation which have been analyzed under other headings in recent editions of *Annual Reviews of Physiology*. Reviews or monographs dealing with special aspects of the subject are appended at the end (309-325).

### ARTERIAL PRESSURE PULSES

Several models for studying the dynamics of the circulation have been described (1, 2). Mathematical analysis suggests that the ratio of pulse volume to stroke volume is determined by the product of heart rate, peripheral resistance, and the reciprocal of the slope of the volume distensibility curves of elastic vessels (3). In a thesis, Carlborg (4) analyzed circulatory disturbances by aid of pressure pulses, pulse wave velocity, etc. Hürthle (5) has analyzed the arterial pulse, ostensibly for physicians. A new optically recording manometer with controllable sensitivity which utilizes capacity changes of a condensor chamber has been described (6). In a following paper (7) aortic pressure pulses of dogs were analyzed. No new facts were established, but certain difficulties in obtaining basal conditions from unanesthetized dogs are indicated in the data. In a series of papers, Hamilton and his colleagues (8, 9, 10) have explored the possibilities of determining systolic discharge of the ventricles by means of arterial pressure pulses. First it was found that, owing to viscous resistance of arteries to rapid stretch, the velocity with which the foot, peak, and tail of a pressure wave is propagated cannot be calculated by classical formulae of tension-length relations established on slowly stretched rings of arteries (8). In addition, the difficulty of estimating the

<sup>1</sup> I am indebted to Gerald Graham for valuable assistance in the collecting of references and in abstracting of some of the material.



diastolic capacity of the arterial reservoir enters into calculations of stroke volume (9). It has been recognized that the time-course of cardiac ejection, as determined by volume curves, is reflected in aortic pulses. But since the form of pressure pulses also depends on the rate at which blood is displaced onward, greater exactness in evaluating the course of cardiac ejection can be obtained by estimating the time relations and magnitude of distention in successive segments of the aorta. The original paper must be consulted for details. Hamilton *et al.* also analyzed the types of ejection in a great variety of pulse patterns frequently obtained in experimental work. With this basic information, judgments regarding the behavior of the left ventricle in experimental clinical studies can be made with more certainty by analysis of central pressure pulses. A number of papers (11, 12, 13), not accessible to the reviewer, dealing with physiological characteristics of arteries, can only be mentioned.

#### ARTERIAL PRESSURE MEASUREMENTS

Improvements in technique for determining arterial pressures in unanesthetized animals have been reviewed recently (14). To these may be added a description of a simplified method for making a pedicled carotid loop (15) and an optical method for measuring systolic pressures in rabbits' ears (16). Warming the rat's tail and applying a 10 mm. cuff close to the base of the tail ensures greater accuracy in the plethysmographic method for determining arterial pressure in rats (17).

Possible pitfalls in the use of the auscultatory determinations of arterial pressures in man are indicated in several reports. Supervention of spontaneous sounds may cause too low recordings of diastolic pressure in fevers (18). Underestimation of systolic and overestimation of diastolic pressure may result when an "auscultatory gap" exists. Its significance has been discussed (19). The occurrence of zero diastolic pressure and tachycardia has been described in some inductees into the armed forces (20). Since this is dynamically impossible, default of the auscultatory criteria under special circumstances is the most probable explanation.

Using direct registration of brachial pressures by a new type of variable condensor manometer, it was concluded that occlusion of a brachial artery elevates systolic and diastolic pressures and increases pulse pressures (ca. 15 mm. Hg.) (21). This agrees in gen-

eral with previous observations on animals (22) but, as pointed out, does not necessarily prove that readings determined by auscultatory method are too high. Agreement was found by Kotte *et al.* (23) between direct and indirect measurements of systolic pressure in normal subjects and in patients with hypertension and aortic insufficiency. However, indirect readings of diastolic pressure were too high, especially in aortic insufficiency. Indirect readings of femoral pressures were accurate only when cuffs wider than those ordinarily used were applied.

Precautions in measurement of human blood pressures have again been stressed. Pressures may be different in the two arms (24). Hyperabduction of an arm may reduce systolic pressure as much as 15 mm. Hg. possibly as a result of stretching or compression of axillary arteries (25). The importance of taking blood pressure readings under basal conditions has again been stressed (26). They are consistently lower than casual determinations, for the latter are affected by physical, emotional, and metabolic activities.

Hillman *et al.* (27) are analyzing medical records of 23,000 officers who have had annual physical examinations over a number of years. Blood pressure values will be correlated with age, weight, mortality rates, and necropsy findings. Since values of 90 mm. Hg. occur frequently in normal subjects at all ages, this may be regarded as the lower limit for systolic blood pressure (28).

*Arterial pressure in childhood.*—Several investigators have followed the changes of blood pressure in children. A gradual increase in blood pressure from an average of 89/60 at six months to a value of 100/67 at four years of age in males, and 93/62 to 100/64 for the same age range in females has been reported. A slight decline in blood pressure occurred during the fifth year of life. There was no significant correlation between blood pressure and body weight (29).

Blood pressure determinations on some 3,000 children from 5 to 16 years of age have been collected and evaluated (30). The children's pressures were followed for fifteen years. Systolic and diastolic pressures increased linearly with each year of age, showing very close agreement between the two sexes. Modal systolic blood pressure ranged from 92 mm. at 5 years to 122 mm. Hg at 16 years, diastolic pressure increased from 52 mm. to 62 mm. Hg for the same age range. It was also found that variations in blood pressure readings for any one individual increased with age. Shock (31)

determined blood pressure values under basal conditions of a small group of adolescent boys and girls. Systolic pressure increased from 103 mm. at 11½ years to 115 mm. Hg at 17½ years in boys, with no significant changes occurring in girls. Beyond 13½ years of age boys showed significantly higher systolic blood pressure. Average diastolic pressure reached a minimum at 14½ years for both sexes and rose continuously thereafter. During adolescence, the average pulse pressure increased 6 to 10 mm. Hg in both boys and girls, and it returned to a preadolescent level at 24 years. Early maturation or growth spurt was accompanied by earlier blood pressure changes. Systolic blood pressure measured at rest was a more reliable measure in selected groups of high school and college students. There was much individual variation from day to day, necessitating repeated readings to establish an average level. Individual variations were great compared to differences among individuals (32).

#### BLOOD FLOW

Technical improvements in determination of blood flow in human extremities are claimed and certain re-evaluations suggested. Optical registration of movements of liquid in capillary tubes are said to represent pure volume changes (33, 40); however, such arrangements represent damped, low frequency systems. A sensitive toe plethysmograph for determining pulse volumes and flow rates was described for clinical use (33). The pulse volume of the big toe varies from .002 to .045 cc. Spontaneous rhythmic variations due to vasomotion are not common. Deep inspiration induces constriction. All vasomotor effects are abrogated by sympathectomy (33).

A water bath temperature of 34°C. was found to give rates of blood flow in the arm which are most comparable with those in covered arms (34). Normal flow rates so obtained are larger than previously suspected (ca. 3.1 cc. per 100 gm. per min.) If generally applicable, blood flow through all skeletal muscles equals 900 cc. per min. instead of 500 cc., based on data of earlier observations (309). However, it has again been demonstrated that blood flow in extremities depends also on body temperature. Thus, the flow through the hands at any temperature (2° to 35°C.) is greater the warmer the body (35). These variations may be very considerable, e.g., ranging from 0.22 cc. to 28.3 cc. per 100 cc. tissue per min. with changes in ambient temperatures from 15 to 38°C. (36).

Measurements of heat loss in a plethysmocalorimeter confirmed previous suspicions that the Stewart calorimeter method is unreliable for determining blood flow because the entering blood has a lower temperature than body tissues generally (36).

By use of plethysmographs of Lewis and Grant with venous compression technique, it was found that fainting and fall of arterial pressure during simulated hemorrhage are accompanied by increased blood flow through muscles, while that through the hand decreases. These statements are predicated on the view that the forearm just above the wrist consists of 60 per cent muscle, an assumption that should be checked. Since the increase in blood flow is reduced by previous nerve block, active excitation of sympathetic dilator fibers is inferred (37). Individuals who faint as a result of anoxia develop an abrupt increase in arm flow (over 10 cc. per 100 gms per min.) as the heart slows and blood pressure falls (38). However, clear-cut evidence is not given as to the extent to which dilatation rather than cardiac slowing is the determinant of the abrupt decline of blood pressure during fainting. The vascular reactions of the nasal mucosa to thermal stimuli applied to local surfaces of the body do not differ in persons resistant, and those susceptible to common colds (39). Attention has again been drawn to inadequacy of customary graphic methods used for determining pulse volume and rate of flow in man (40). In general, the critique of Wright & Phelps (41) is endorsed and, with the exception of the recorder, their procedure is recommended. Errors can also be introduced in calculation of results in determining flow for one minute intervals. This can, however, be avoided by an expedient adopted by Eckstein *et al.* (42) who adapted the Wright and Phelps technique to the study of the circulation in dogs during hemorrhagic shock. Decrease in flow out of proportion to the drop in blood pressure was the outstanding feature in irreversible circulatory failure.

Hertzman *et al.* (43) have made a first approach toward calibration of their photoelectric plethysmograph in terms of cutaneous blood flow through establishment of flow equivalents of "filter units." The smallest value thus obtained for phalanx blood flow (0.048 cc. per cu. cm. per min.) is still ten times the average for basal skin flow determined by the modified Hardy-Soderstrom method (44). By the latter method it was found (45) that the average basal flow at 27°C. is essentially the same (about 73 cc. per sq. in. per min.) in older male subjects as in younger ones. Peripheral

blood flow is within normal ranges during heart failure, but improves as a result of strophanthin K and digitalis alkaloids, intravenously administered (46). However, it is less in hypertensives (about 55 cc. per sq. in. per min.) who show greater flow rate in the upper and smaller rates in the lower parts of the body (47). Patients who benefited most from splanchnic resection or the Smithwick operation showed a decrease in average peripheral blood flow, also a fall of temperature in the upper and a rise in the lower parts of the body (48). In this connection it is not wholly clear whether differences in skin temperature reported during hypertension and following operations might not represent differences in vaporization rather than in blood flow in the upper and lower portions of the body.

Other devices for studying blood supply of the skin have been described. These include an instrument for measuring the quantity of blood in the web of the hand and its degree of oxygenation (49), a hot wire and thermocouple for recording surface pulsations (50), also methods for determining circulation time by injection of radioactive phosphorus corpuscles (51) or fluorescein sodium (52) and by use of the oximeter (53). A method for determining reduction time of cutaneous blood after cessation of blood flow through a pressure cuff has been introduced (54). Its usefulness as a means of evaluating bodily fitness has been tested (55). By use of corpuscles labeled with radioactive phosphorus, it was found: (a) that incomplete emptying of the ventricles increases circulation time and mixing time; (b) that no evidence of any significant depot function can be detected by injection of epinephrine or performance of heavy work; and (c) that one lung (abnormal state) holds 16.7 per cent of the circulating blood volume (56). The thermister—an electrical unit, the resistance of which changes exponentially with the reciprocal temperature—was not found to work as an accurate rheometer *in vivo* (57).

A serious attempt has been made to devise a more accurate thermocouple flowmeter through such expedients as mounting the thermocouple in a cannula, improving its time characteristics, etc. The conclusion is reached that thermal methods are likely to remain unsatisfactory owing to such basic limitations as: (a) uneven temperature in various parts of a bloodstream; (b) long thermal lag of the fluid surrounding the heated element; (c) lack of linearity between temperature changes and flow; and (d) occurrence of

backflow (58). The structures responsible for intermittency of flow in mammalian tissue, frequently described, have been restudied in wings of bats (59). The most common activity consists in rhythmic alternation of constriction and relaxation of entire networks of minute vessels; more rarely individual arterioles or capillary sphincters exhibit independent cyclic vasomotion.

*Cerebral circulation.*—Some recent developments in the study of the cerebral circulation were given in Volume VIII of these reviews (60). Since that time a new method for estimation of cerebral blood flow in man has been described by Kety & Schmidt (61). It is based on the principle that the volume of blood flowing through the brain determines the rate at which cerebral venous blood acquires the same content of nitrous oxide as arterial blood. Its quantitative reliability requires the assumption: (a) that different types of brain substance and fluids represent a homogenous tissue, as far as blood flow and nitrous oxide capacity are concerned; (b) that a sample from an internal jugular vein represents mixed venous blood; (c) that the solubility ratio of nitrous oxide in blood and brain is reasonably constant; and (d) that the gas itself does not alter the cerebral circulation. The principle of the method was subjected to searching mathematical analysis and the assumption tested as rigidly as possible by comparisons with actual measurement of cerebral flow. Average values of blood flow for human brains equaled 62 cc. per 100 gms. per min., which compares favorably with directly measured flows of 47 cc. per 100 gms. per min. for brains of monkeys (62). Cerebral oxygen consumption in both was 3.7 per 100 gms. per min. Similar values were obtained independently by another method (63), viz. an average flow of 617 cc. per min. and average oxygen consumption of 39.2 cc. per min. (average human brain weight, 1,360 gm.) The procedure consisted in injecting Evans blue into the right internal carotid artery and simultaneous sampling of blood from the left internal jugular vein and femoral artery. By use of Gibb's thermoelectric method it was found that only a passive slowing of cerebral blood flow occurs with the fall in arterial pressure evoked by labyrinthine stimulation (64). Several communications (65, 66) deal with effects of hypotension, hypoxia, and carbon dioxide on cerebral blood flow and metabolism.

*Renal blood flow.*—Anatomical studies of the vascular arrangements in the kidneys of several species by means of neoprene in-

jections revealed evidence of vessels shunting the glomeruli (67). No opinion was expressed as to whether these communications are sufficiently numerous to be of dynamic significance. Two direct methods for recording renal blood flow in anesthetized animals have been described (68, 69). By this means it was found that the fall of blood pressure which follows release of a limb tourniquet causes a greater reduction in renal flow than a similar decline due to hemorrhage (70). The convenient method of determining renal blood flow by dividing the amount of an excretory substance (e.g., *p*-aminohippuric acid) excreted per minute by the amount which the kidney extracts from one volume of unit blood or plasma has been retested as regards its serviceability in the study of hemorrhage and shock. Phillips *et al.* (71) found it dependable during sudden hemorrhage provided arterial pressure remained above that required for measurable excretion (about 50 to 60 mm. Hg). In bled pentobarbitalized dogs, a primary constriction of efferent arterioles was followed by a subsequent constriction of afferent arterioles. When hemorrhage surpassed a certain limit, when blood lost was not replaced for hours, or when muscle trauma exceeded a certain limit, estimated renal blood flow fell to zero levels even when arterial pressure was still 80 to 100 mm. Hg. Selkurt (69, 72) compared renal blood flow calculated from *p*-aminohippurate clearance with simultaneously measured direct flow. In pentobarbitalized dogs, the former averaged 91 per cent of the latter. Following a twenty-minute period of ischemia indirect determinations of renal blood flow indicated a greater reduction than actually existed (indirect being 46 per cent of direct) (69). During hemorrhagic hypotension (60 to 40 mm. Hg) considerable renal flow occurred when clearances were zero. After reinfusion of blood following a prolonged hypotension, direct renal flow returned to 67 per cent of control values, whereas indirect readings averaged only 39 per cent of the direct blood flows (72, 73). Obviously, indirect methods are unreliable in estimating the beneficial effects of blood infusions on renal flow. Determinations of renal blood flow by indirect clearance methods in patients with chronic congestive failure (74) indicated that renal blood flow is reduced to 20 per cent of normal, while cardiac output is decreased not more than 50 per cent. Since no correlation exists between venous pressure and reduction of renal flow, the failure of renal system is not accounted for by the back pressure theory.



*Pulmonary circulation.*—Experiments on excised lungs (75), roentgenographic observations on animals (76, 77), confirmed by histological studies, substantiate the generally accepted view that lung inflation with its expansion of air cells acts to increase the vascular capacity of the pulmonary vessels. The view that this is a significant propulsive force in moving blood through the lungs requires further study.

The difficulties of obtaining unequivocal evidence for existence of pulmonary vasomotor nerves led Daly *et al.* (78, 79) to devise a perfused "whole animal preparation" in which the two circulations were supplied with separate rhythmic pumps. Bronchoconstriction, when complete, affects the degree and direction of pulmonary arterial pressures variously, depending on its rate of onset which determines the final position in the respiratory cycle in which lungs are immobilized. Partial bronchoconstriction affects pulmonary arterial pressures through changes in the direction of the mean air volume of the lungs. Since stimulation of the cervical vagosympathetics may produce concomitant bronchomotor and esophageal actions, the changes in pulmonary blood pressure give probable rather than quite certain evidence for operation of pulmonary vasomotor fibers (78, 79). The bronchial flow in dogs (11 to 20 kilo), measured by a bubble flowmeter, is not large (about 2 to 8 cc. per min.). Bronchial vessels are controlled by vagal dilators and sympathetic constrictors (80).

Methods for inserting a catheter via the right heart into the pulmonary artery of dogs (81) and of man (82), and optical registration of calibrated pressure pulses have been described. Data so obtained should be evaluated cautiously. Inspection of published records suggests that an overdamped system was used in recording curves from dogs (81), and that artifacts complicated some of the records obtained from man (82).

The subject of pulmonary edema has recently been discussed by Drinker (311). The difficulty of producing experimental pulmonary edema by large saline infusions can be overcome by its infusion under high pressure into a central common carotid artery (83). It is suggested (but not proved) that carotid-pulmonary reflexes operate which either dilate pulmonary vessels or produce capillary permeability changes.

*Portal circulation.*—The mean pressure in the portal system during expiratory pauses averages about 12 mm. in anesthetized

dogs (84). These values are a trifle lower than those reported in man (85). Large changes in portal flow can occur with only 2 mm. Hg difference in portal-caval pressures. The volume flow through the human liver has been estimated at about 1,500 cc. per 1.73 sq. m. per min. by an indirect method (86). The rate at which bromsulphaline was removed from blood by the liver, its concentration in blood afferent to the liver, and its concentration in blood leaving the liver were determined, and hepatic flow calculated by use of the Fick principle (86).

#### PERIPHERAL RESISTANCE

In hemodynamic studies the adjective, "peripheral," refers solely to distal branches of the arterial tree, regardless of whether these are located in the interior or surface of the body. To judge from many writings, physicians and even some physiologists seem to have difficulty in maintaining correct usage of the term. Thus, a physiologist writes, "the peripheral resistance is increased but the internal resistance is reciprocally reduced." It is charitable to omit references to similar misuses of the word, and the confusion in thinking to which it gives rise. The term, "peripheral resistance," includes all factors which impede the flow of blood from branches of the arterial tree and, according to Poiseuille's law is proportional to characteristics of the tube ( $8L/\pi R^4$ ) and the blood viscosity ( $\eta$ ). Lamport (321) has suggested the term *hindrance* for the tube factors.

The adequacy of Poiseuille's law, which is basic in estimations of peripheral resistance, has been questioned repeatedly. The fact that reduction in viscosity of blood and structural defects create turbulence—recognizable as clinical murmurs—indicates that laminar flow is maintained by a small margin. Murmurs caused by structural defects can be abolished in an artificial circulation apparatus by increasing the viscosity of blood (87). Vibrations can also be recorded from normal arteries under experimental conditions. However, this does not necessarily prove the existence of turbulent flow, for tense elastic tubes may themselves undergo transversal vibrations (88). New proof has been reported for the existence of streamline flow in the arterial system of cats and dogs (89), and in the portal system of dogs (90).

Aside from viscosity, the diameter of smaller branching vessels is the chief factor which modifies peripheral resistance. As a first

approximation, these are generally considered to be constant in size during pressure changes in formulations such as those of Poiseuille, Bingham, and others. Actually this is not the case in the body. The mean diameters of blood vessels down to arterioles, and possibly beyond, are changed passively by alterations in mean internal pressures and possibly by alterations in the contours of pressure waves. It is also generally stated that diameters are influenced by pulse pressure, but Green *et al.* (91) could establish no consistent relations. This deserves further study. Rashevsky (88) properly points out that in the case of distensible tubes, such as blood vessels, the size and shape of the boundaries are themselves determined by distribution of velocities and pressures which, in turn, are affected by boundary conditions. The complicated integrodifferential equations required to state these interactions cannot be solved at the present time. We must remain content to use approximation methods of mathematical biophysics and experimental methods. Unfortunately, results obtained by studying vessels in the body can never be quite conclusive, owing to the fact that small vessels also undergo periodic variations in size due to vasomotor activity. This has been observed repeatedly in vessels of many regions. In relating peripheral resistance to size of vessels it is probably more accurate to speak of net or mean diameters.

*Regional or territorial resistances.*—By these terms are understood the resistance offered to flow through an organ or territory. A study of pressure-flow relations of organs or isolated tissues at different perfusion pressures has served to evaluate mechanisms concerned in peripheral resistance. In order to interpret results, care must be taken to exclude collateral circuits (92) and to recognize the vascular arrangement of organs so perfused. In order to simplify analyses small arteries and arterioles are generally treated as though they branched dichotomously until capillaries are reached. Resistances are estimated from the respective lengths and diameters of successive linear segments. It now appears questionable whether such vascular arrangements are dominant in the viscera. Larger arteriovenous shunts have long been familiar to anatomists, but the existence of smaller arteriovenous connections from which true capillary shunts arise seem to dominate in the abdominal viscera (93). In some organs, such as the spleen, liver, and heart, vascular sinuses exist, the control of which is still not fully established (320). The possibility must be kept in mind that

such arteriovenous communications and sphincters rather than arterioles leading into capillary pathways constitute the true stop cocks of the circulation which dominate changes in peripheral resistance in these organs.

The viscosity factor which enters into Poiseuille's equation apparently varies independently of the shearing force determined in other ways; it decreases even in rigid tubes less than 0.3 mm. in diameter, and at subcritical pressures and rates of flow, it decreases rapidly in still smaller tubes (315). When whole blood the viscosity of which has been determined *in vitro* is perfused through organs its viscosity calculated by Poiseuille's law becomes progressively less with increasing heads of pressure. For this reason the term *effective viscosity* has been introduced. This is apart from natural changes in blood concentration that may result from variable loss (or gain) of plasma fluid—particularly in secretory and excretory organs—in its passage through capillaries. Furthermore, when perfusion of an organ is started, a certain critical minimal pressure must be applied before blood starts to move. This is the "yield pressure." In the kidney within the body 14 mm. Hg seems a better value than 20 mm. Hg commonly used (94). A number of interpretations for this phenomena have been made. A certain degree of corpuscular packing and deformation of corpuscles may be required to start a flow. In other words, the blood may be converted into a plastic material and moves as such at a slightly supercritical low pressure. The blood may not change from a viscous to a plastic fluid, but a yield pressure may be required to overcome an elastic resistance between corpuscles and capillary walls (315). This concept is plausible whenever it may be assumed that the bulk of blood flows through true capillaries rather than small arteriovenous circuits, for the diameter of blood cells is approximately the same or less than that of the capillaries. Despite uncertainties and apparent limitations which seem to exist with regard to the applicability of Poiseuille's law, it continues to serve investigators as a guiding beam in the study of changes in peripheral resistance, flow, and viscosity.

Most investigators had found that after a flow has once been started a linear relation exists between pressure difference and flow. However, Green *et al.* (91) failed to find such a relationship in skeletal muscles. They explain the curves which run convex toward the pressure axis by the fact that successive vessels determining

resistance dilate passively as pressure increases, and/or that new capillary paths perhaps open. This view is supported by studies of kidneys perfused with pectin solution (94). Owing to this lack of straight line relationship of pressure to flow at different pressures, these investigators caution against attempts to interpret vasomotor changes in organs or the body as a whole from computations of resistance from pressure-flow relations when aortic pressure changes materially. By integrating aortic, portal, and inferior vena caval pressure curves during expiratory rest, extravascular influences can be discounted and mean values of hemodynamic significance obtained (84). From such analysis it was concluded that hepatic resistance to flow is about one-eighteenth that of all tributaries to the portal system; this compares with older estimates of about one-ninth (95). By comparing aortoportal and portal-caval gradients at the same moments it was concluded that portal resistance increases relatively more than mesenteric resistance during hemorrhagic hypotension and circulatory failure following reinfusion of blood.

Opdyke (96) evaluated directional changes in total resistance in all aortic branches below the diaphragm by compressing the lower thoracic aorta for a few seconds while the decline of femoral pressure was recorded optically. The time required for pressure to fall between standard fixed levels under different experimental conditions gives an estimate of changes in territorial resistance in the abdominal viscera and legs, provided variations in collateral flow are prevented and it is assumed that no functional alterations occur in the volume-elasticity relations of the arterial tree beyond the compression. Evaluated by this method partial cerebral ischemia induces intense increase in resistance sufficient at stabilized arterial pressures to reduce peripheral blood flow materially. Persistent cerebral ischemia (2 to 3 hrs.) leads to gradual reduction of resistance and circulatory failure resembling irreversible shock produced in other ways.

The kidney is an organ in which two sets of capillaries are placed in series and an enormous increase in viscosity occurs at higher pressures due to augmenting glomerular filtration. While the kidney is therefore not a good organ in which to study the validity of Poiseuille's law, it is important to know the pressure flow relations which obtain in the body under various states of arterial pressure. Selkurt (94) investigated these pressure flow re-

lations at different aortic pressures. A clamp applied to the aorta just above the renal arteries was tightened sufficiently to produce stabilized lower arterial pressures and stabilized venous renal flows below the constriction for about ten minutes. By determining pressure-flow relations at various pressure levels it was found that the plotted curve is not a linear but an exponential one, concave toward the pressure axis. Apparently renal resistance at different aortic pressure differs from that found for the leg (91) in which curves are convex toward the pressure axis. Changes in viscosity due to glomerular filtration theoretically explain these differences.

Since Poiseuille's equation contains separate factors for viscosity ( $\eta$ ) and for changes in caliber and length of vessels ( $8L/\pi R^4$ ), it is often desirable to separate their effects. While this can easily be done on paper, its experimental realization has not been entirely satisfactory. Probably no viscous fluid can be made to flow through living capillaries without some interchange of liquid with tissue spaces. Consequently, the viscosity of capillary blood and tissue pressure both alter. In the case of blood, the red corpuscles also change in size as a result of respiratory interchange of gases. Constriction of arterioles probably involves not only primary decrease in caliber but also a decrease in capillary pressure which in turn alters viscosity and tissue pressure relations.

Despite these complexities of the problem some progress has been made. By use of an isolated whole leg muscle and skin preparation, Green *et al.* (91) attempted to evaluate changes due to vasomotion by comparing pressure-flow (P/F) relation under experimental conditions with those of previous controls in the same animal. Control pressure-flow (P/F) curves were made over a large range of pressures under conditions in which complications of viscosity and collateral circuits could be fairly well controlled, and the relations were plotted as a curve. With this, a curve similarly constructed during experimental procedures could be compared. Since this is a laborious procedure and precludes establishment of rapid changes, the more useful expedient was adopted of comparing the P/F relation at any existing pressure on the control curve. While the applicability of the method is somewhat limited, it served a useful purpose in demonstrating increased vasomotor action at the summit of Traube-Hering waves and following changes in vasomotor activity of the leg in hemorrhage (97). Progressive vasoconstriction in renal vessels during hemorrhage has also been

demonstrated by use of the same principle (94). Furthermore, the autonomous control of renal flow during variations of arterial pressure does not require postulation of a special intrinsic nervous mechanism; this is well taken care of by physical changes in viscosity due to different rates of glomerular filtration at different pressure heads.

*Total peripheral resistance (T.P.R.).*—This term, translated from the German, *Gesamtwiderstand*, signifies the resultant resistance to runoff from numerous parallel circuits developed in the aorta. Since under stabilized conditions the efflux from the various branches of the aorta equals the cardiac output it can be calculated as mean pressure divided by cardiac output.<sup>1</sup> The total resistance is less than that of the territorial resistances for, as in electric circuits, its reciprocal equals the sum of the reciprocals of various parallel resistances.—

$$1/TPR = 1/R^1 + 1/R^2 + 1/R^3 + 1/R^4 \dots \text{etc.}$$

Calculations of T.P.R. enable us to differentiate whether changes in arterial pressure are due to cardiac, or to a summation of many peripheral factors which affect resistance. Since T.P.R. is the resultant of resistances in numerous parallel circuits a directional change does not necessarily indicate a similar directional change in all territories; resistance may in fact increase in some organs and decrease in others. Since caliber changes due to vasomotion are prepotent in comparison with all other determinants, such as viscosity, passive changes in size of blood vessels, tissue, capillary, and venous pressures, it seems probable that comparisons of T.P.R. at different levels of arterial pressure indicate the trend, but not necessarily the magnitude of vasomotor activity in dominant territories of the body. However, Green *et al.* (91), on the basis of studies on the dog's hind limb, concluded that the possibility exists that changes in T.P.R. may not always indicate the trend of vasomotor changes when arterial pressures alter simultaneously. As they recognized, it is questionable whether deductions drawn from pressure-flow curves of the leg can be applied to the body as a whole. Indeed, such application requires the dubious assumption that no vasomotor changes occurred in their leg preparations (cf. page 268) and that similar pressure-flow curves pertain to circulations in other organs. They obviously must be quite different in

<sup>1</sup> This obviously assumes a zero venous pressure which, in view of the limitations to be discussed later, suffices for calculations of T.P.R.



the heart and have been found to be so in the kidney (94). While it is my considered opinion that changes in T.P.R. in the presence of alterations in arterial pressure indicate the directional changes of dominant vasomotor changes, the fact that T.P.R. can be expressed in numerical units perhaps gives a sense of quantitation which does not exist.

Seligman, Frank & Fine (98) endeavored to determine the hemodynamic consequences of altering blood viscosity. Assuming that peripheral resistance ( $R$ ) per kg. dog is unity, they write Poiseuille's equation,  $K = O \times \eta / P$ ; in which  $O$  = cardiac output in cc. per min. per kilo,  $\eta$  = apparent viscosity, and  $P$  = mean arterial pressure. In normal dogs,  $K$  ranged from 4.4 to 14.8 (av. 8.1 in 70 per cent of animals). By writing  $R = P \times 8.1 / O \times \eta$ ,  $R$  gives a rough expression of the ratio of peripheral resistances (apart from viscosity) in different animals. They found that raising corpuscular concentration by venovenous exchange of packed cells for whole blood, normal dogs maintained a constant T.P.R. by lowering peripheral resistance. But when the hematocrit reading was raised by arteriovenous exchange of packed cells for whole blood, the peripheral resistance was increased. The legitimacy of certain mathematical hurdles used in the application of Poiseuille's equation to determine T.P.R. without involvement of viscosity may be questioned. Utilization of a value involving the weight of the dog and adoption of a value of 8.1 for  $K$  when actual values show great variation is probably too rough a maneuver, particularly since data from individual experiments indicate that the values for  $K$  appear to be suspiciously related to cardiac output.

*Units of resistance.*—By definition peripheral resistance is the ratio of pressure to flow. It can be calculated in c.g.s. units or expressed as arbitrary units, provided values for mean arterial pressure and flow are known. In the calculation of T.P.R. cardiac output per second or per minute equals the rate of flow from the aorta under stabilized conditions.

At a meeting of the *Circulation Group* associated with the *Federation of American Societies for Experimental Biology* (March 14, 1946), the advantages and disadvantages of current methods for expressing peripheral resistance were frankly discussed. The chief difficulty in the employment of present units is that, while suitable in one field of study, they are unreasonably small or large when applied to other fields. While no agreement as to the most

universally satisfactory units emerged, a brief summary of the chief arguments may here be placed on record.

The oldest and most widely used formula for calculating T.P.R. reduces all values to c.g.s. units.  $TPR = \text{mean arterial pressure} \times 1,332 / \text{cardiac output in cc. per sec.} = \text{dynes. sec./cm.}^5$  The writer called these absolute units (a.u.), but owing to their small magnitude a large absolute unit (A.U.), equal to 1,000 small units, would be more useful in the study of territorial resistance (cf. use of small and large calories). Continued use of these conventional units was recommended by the writer because they have been in use for a long time and a change might be confusing. However, it has been pointed out that nothing is gained by the unnecessary multiplications involved. Green *et al.* (91) in the study of territorial resistances suggested as a unit, *mm. Hg mean pressure flow in cc. per min.*, called a P.R.U. This unit, however, is so large that T.P.R. of man would be approximately 1/60 P.R.U. It was suggested that by substituting cc. per sec. a value of unity would be achieved. Since resistance calculated by any of these methods varies inversely with size of the body, the introduction of a factor for body weight or surface area has been suggested. An arbitrary formula,  $R = 3 \times \text{mean arterial pressure} / \text{cardiac index}$ , which gives normal values of 100 in man was suggested by Bazett *et al.* (99). H. C. Wiggers (100), however, found no good correlation between T.P.R. and either size or surface area in dogs. Perhaps this is explained by the fact that pressure-flow ratios are related to the number of small vessels in each of the main parallel circuits and the rate of irrigation in each, which is not necessarily related either to body weight or surface area (101).

Another difficulty in the use of current units is the apparently paradoxical fact that the resistance of the whole (TPR) is less than that of its parts. The use of the word "total" which implies summation has been objected to for this reason. This is obviated by use of a reciprocal unit, flow divided by pressure, which roughly expresses conductance. Such a unit would have the dimensional formula,  $M^{-1} L^4 T$ , from which convenient units can be derived. Gomez (101) suggested the expressions,  $\text{Flow/Pressure} = \text{cm.}^3 \text{ per sec./gm. per cm.}^2 = \text{cm.}^3 \text{ per min./80} \times \text{mean arterial pressure}$ . Either of these yields numerical values approximating unity. A satisfactory unit suitable for all purposes still remains to be suggested.

## NERVOUS CONTROL OF THE PERIPHERAL CIRCULATION

Attention may be called to comprehensive reviews of literature dealing with the nervous control of the circulation in recent issues of these Reviews (102, 103) and also to the portion of the chapter on the visceral functions of the nervous system in this volume which deals with the innervation of the vascular system. Heymans *et al.* (104) have discussed the role of the bulbar vasomotor centers in the light of new evidence.

The vasomotor reactions elicited by stimulation of the carotid body and sinus have been more carefully studied. It is quite generally accepted that afferent impulses evoked from pressoreceptors exert a reflex inhibitory action (slowing of the heart and vasodilation), while those released by chemoreceptors in the carotid body exert excitatory reactions (increased heart rate and vasoconstriction). This is supported by investigations indicating that asphyxia and anoxia increase the amplitude of spikes recorded from the splanchnic nerves, and that this response is abolished by section of the buffer nerves (105). The general opinion that in dogs the efferent pathway is entirely in thoracolumbar sympathetics has been confirmed by several investigators (106, 107). However, reflex vasodilation can still be evoked in completely sympathectomized cats via cholinergic dorsal root fibers (108).

The reflexogenic changes in blood pressure are usually attributed in part to alterations of total peripheral resistance induced by generalized vasomotor changes. This concept requires refinement. During perfusion of the salivary glands and hind limbs of dogs under constant pressures, chemoreflexes induced by sodium cyanide elicit a primary vasoconstriction followed by dilatation, while sodium carbonate caused vasodilation only (106). Hypoxia basically causes reflex constriction which develops its maximum in the mesenteric vessels more slowly than in the limbs. By comparing the ratios of volume flows before and during such stimulation one finds that the relative intensity of reaction is greater in the limbs than in the intestines; indeed, the latter never constrict to their full capacity (109). It would therefore seem that the dominant resistance in chemoreflex reactions is not in the splanchnic area. The nature of the response suggests that the vasomotor reactions serve to redistribute volume flow. Differences in relative blood flows through mesenteric, renal, and limb vessels also follow use of epinephrine, prinine, and priscol (110).

Experiments in which cardiac output was determined and T.P.R. was calculated revealed that the reflex pressor effect during central vagus stimulation is dominantly due to increase in T.P.R. However, the depressor effect following sinus nerve stimulation or spinal cord transection is primarily due to decreased cardiac output and only slightly to reduction in T.P.R. The reduced cardiac output was attributed to reduction in venous return secondary to dilatation of postarteriolar vessels and pooling of blood (111). One wonders whether cardiac slowing, which usually accompanies stimulation of the sinus nerve, was eliminated as a factor.

At various times it has been claimed that limited vascular reflexes arise which affect the blood supply in one locality or organ. Malinow *et al.* (112) were unable to confirm the operation of a pulmonary-coronary reflex in pulmonary embolism. Riser *et al.* (113) could not demonstrate a direct reflex effect of carotid sinus stimulation on retinal vessels.

Hypersensitivity of the carotid sinus presents a definite clinical problem. Its development after ligation of common and internal carotids has been reported (114). Nathanson (115) believes that the hyper-reactive reflex is due to hypersensitivity of the vagus nerve. While stimulation of the carotid sinus is usually harmless, the report of seven cases of contralateral hemiplegia suggests caution in such practice in elderly persons with arteriosclerosis (116).

#### VENOUS PRESSURE AND FLOW

*Peripheral venous pressures.*—In view of small differences in venous pressures that may need to be evaluated, establishment of an accurate zero reference level becomes of importance. Technically, it has not been established whether a level at the base of the right atrium or, as Cournand (117) has suggested, at the apex of the right ventricle is dynamically more correct. According to Winsor & Burch (118) at least nine different reference levels are in use in determination of peripheral venous pressures in man. They (119) suggest use of a reference zero which is easily determined and applicable to recumbent and upright sitting positions, viz., the junction of two planes, one transverse through the thorax at the level of the fourth intercostal space adjacent to the sternum, the other longitudinal midway between anterior and posterior surfaces of the thorax at the xiphoid process. Static and dynamic studies indicate that peripheral venous pres-

tures measured in arm veins are determined chiefly by tissue pressures when they are collapsed, and only reflect changes in central venous pressures when they are distended (120). The average normal peripheral venous pressure measured in the median basilar vein ranges from 50 to 140 mm. H<sub>2</sub>O in the supine position and 92 to 145 mm. H<sub>2</sub>O in the sitting position (119). Slight rise and fall of pressures have been noted during expiration and inspiration respectively (119, 121). Small sex and diurnal variations observed are probably fortuitous and of no significance. Curiously, abdominal compression appears to decrease cubital venous pressure (119). Increase in intrapulmonary pressure during various types of positive pressure breathing raises peripheral venous pressure in a predictable manner (121). However, owing to mechanical conditions, peripheral venous pressure does not increase or decrease proportionately to intra-atrial pressures when intrapulmonary (and intrathoracic) pressures are altered (122). The pressure gradient between the femoral vein and atrium is decreased by elevation of intrapulmonary pressure (the atrial pressure being raised markedly, the femoral pressure less so) and increased by reduction of intrapulmonary pressure (the atrial pressure being reduced markedly, the femoral pressure less so). Venous pressure gradients are not necessarily an index of the rate of venous return; they depend also on blood viscosity and diameters of tubes, including partial collapse of veins entering the thorax (122). Winsor *et al.* (119) include a good diagram showing venous pressure gradients in man from the dorsalis pedis to the chest veins (gradient 81 mm. H<sub>2</sub>O), and similar values (80 mm. H<sub>2</sub>O) are included in Holt's report on anesthetized dogs (122). In congestive heart failure venous pressure gradients are usually reduced but pressures are not necessarily increased in the cubital vein. Elevations to 250 mm. H<sub>2</sub>O are always accompanied by other obvious clinical signs. Expedients have been suggested for utilizing venous pressure measurements to determine venous thrombosis in the legs (123), to differentiate edema of cardiac and hepatic origin (124), and to estimate blood flow through the upper extremities (125).

*Central venous pressures and venous flow.*—The phasic cardiac variations of flow in the external jugular vein of dogs have been recorded by a bristle tachograph and correlated with cardiopneumatic variations (126). This subject is extensively discussed in Groedel's monograph (317).

It is generally believed by physiologists on the basis of experiments performed on heart-lung preparations and controlled circulation animals that up to the point of decompensation cardiac output is determined by atrial or central venous pressure (127). With the study of human subjects in whom atrial catheterization has been performed this concept has been both confirmed and denied. (a) In moderate bleeding which causes a fall of central venous pressure, it is reported that cardiac output decreases (128), also that it remains unaffected or even increases—in one case 50 per cent (129). (b) The large increase in cardiac output in chronic anemia is accompanied by an increase in atrial pressure (130), or by no significant change (131). (c) Anxiety increases cardiac output without a rise in central venous pressure (132). (d) Digitalis decreases the central venous pressure and reduces cardiac output in normal subjects, while it increases it in cardiac failure (133). (e) Exercise which presumably increases venous return causes a fall of central venous pressure (134). In cardiac failure, central as well as peripheral venous pressures are elevated and the gradient becomes less (135). The concept that this is due to "back pressure" has recently been challenged (136, 137), the alternative view being put forward that the rise in venous pressure is due to a preceding increase in blood volume (137). However, simple plethora produced by rapidly transfusing whole blood (5 per cent of body weight) results only in a transient increase in atrial pressure in dogs (134). Induced muscular contractions while atrial pressure is still high cause a decrease in central venous pressure, as it does in normal dogs. Myocardial depression following ligation of coronary arteries, atrial fibrillation, etc., does not increase atrial pressure at rest, but does produce a slight rise after exercise. The concept is advanced that hypervolemia is advantageous in cardiac disease because it permits compensation for repeated depletion of effective circulatory volume to critical levels during any exertion great enough to tax the heart's competence (134).

We may with reason be proud of our achievements both in the development of accurate pressure recorders and in the evolution of catheterization techniques which have made such instruments adaptable to human studies of intracardiac pressure. However, since different groups of investigators are not in agreement regarding directional changes of atrial pressures in similar clinical conditions (see above) and since some of the interpretations of myocardial behavior do not square with those derived from physiologi-

cal studies, it is pertinent to examine the possibility that clinical registrations of atrial pressures need further refinement. Failure to recognize or an unwillingness to admit existence of pitfalls constitutes one of the most serious impediments to progress; frank recognition and admission that technical inaccuracies exist lead to further efforts to eliminate or evaluate them, and is a forward step in the pathway of medical progress.

A number of minor errors may possibly affect human atrial pressure determinations. Meticulous attention to small step calibration in relation to a base line is obviously important; pressures are not always recorded linearly by metal or rubber membrane manometers. Determination of a zero even with fluoroscopic control is only approximate; postmortem checks on dogs indicate that variations of 10 mm. saline levels in the closed chest can occur. Moreover, flexible catheter tips are apt to change their position through cardiac or respiratory movements; indeed, sometimes they need to be pushed in or pulled out in order that records may be obtained. The common practice of using small irrigations of citrate between recordings may inadvertently result in the introduction of sufficient volumes to affect venous pressures, particularly since citrate depresses the myocardium slightly. A summation of such small errors may conceivably be of importance, especially in types of clinical investigations in which control determinations of atrial pressure cannot be made in the same subject and in which reliance must be placed upon average normal values.

There are, however, greater possibilities of error in the measurement of recorded curves and in the evaluation of changes in intrathoracic pressure. The measurement of optical records of central venous pressures presents some difficulties. These have been discussed in several recent papers (84, 138). The mean atrial pressure, especially when recorded by insensitive manometers, is of doubtful dynamic significance, since it is dominated by respiratory variations in intrathoracic pressure. A mean of cardiac pressure variations determined during a cycle at the end of expiration is probably of greatest dynamic significance. It certainly avoids extravascular effects of deep breathing which are dynamically unimportant, but unquestionably dominated results reported by a number of investigators cited above.

Since the heart and large veins within the thorax are under variable subatmospheric pressure, atrial pressures recorded by



pressure recorders balanced against atmospheric pressure do not give expression to the true atrial pressure available for ventricular filling. This is given by the effective venous pressure (E.V.P.) calculated as the algebraic difference between intrathoracic and recorded venous pressures, both measured during expiration. The possibility of achieving estimates of effective venous pressures in man are increased as a result of new techniques described by Bloomfield (139). The influence of changes in intrathoracic pressure on correlation of actual venous pressures and cardiac output are well illustrated in experiments of Holt, quoted above (122). Marked increase of central venous pressure due to increase in intrathoracic pressure was accompanied by a 33 per cent decrease in cardiac output, whereas marked reduction of central venous pressure by decrease in intrathoracic pressure resulted in a 13 per cent increase in cardiac output. It can be predicted that effective venous pressures would correlate with cardiac output changes when direct venous pressures show opposite trends.

While the principle of calculating effective venous pressure by differences in intrathoracic and intra-atrial or caval pressures is sound, the question has been raised whether measurements of intrathoracic pressure around the heart and large arteries can be made accurately by conventional methods (140). Boyd & Brookhart (141, 142) believe that imperfect transmission of pressure across the pericardium may be a factor, but of this the reviewer remains to be convinced by more substantial evidence.

*Venous return.*—The influence of subsidiary factors supposed to aid venous return has been reinvestigated. The conception of Franklin (324) that inspiratory constriction of the inferior cava by the diaphragm and caval band may reduce blood flow is not supported by flow measurements in "open chest" experiments; blood flow is always augmented with descent of the diaphragm (147). In a series of papers by Duomarco *et al.* (143 to 146), the view that respiratory movements aid venous return is not supported. Unless the inferior cava is greatly distended, a block occurs at the diaphragm, by virtue of which pressures are suddenly reduced and become oscillatory above the diaphragm (143). Experiments on artificial models indicated that the phenomena of venous collapse described by Holt (148) for branches of the superior cava also cause an inferior caval block. This phenomenon would make it physically impossible for variations of intra-abdominal and intra-

thoracic pressures to influence venous return directly. It may be questioned whether the condition of their animals was entirely normal for: (a) they found that the block disappeared when the inferior cava was distended; (b) other observers (84) have been able to record cardiac variations of venous pressures below the diaphragm; and (c) it is known that they can be recorded as normal liver pulses in man. Also in conflict with these conclusions are the observations (149) that the decrease in intrathoracic pressure caused by ventricular systole is a force which aids return of small amounts of additional amounts of blood into the chest. However, since a part of the energy so created is expended in decreasing the volume of the chest and in aspirating air into the lungs, the magnitude of this force is much less than formerly believed. Two communications present an academic discussion of factors promoting return of blood to the heart (150, 151).

#### HEMODYNAMICS OF SHOCK

The general advances in our knowledge of shock during the war years were analyzed by Gregersen (316) in a preceding volume (see also 152, 153). Evidence was discussed which favored the conclusion that loss of circulating fluid is the primary factor in causation of shock and that generalized capillary leakage as well as toxic and nervous influences are of subsidiary importance and enter only under special circumstances.

"The reduction of blood volume occurs at the time of injury. . . . Hence, contrary to earlier concepts, the gradual failure of the circulation at least in these two forms of shock (traumatic and hemorrhagic) cannot be ascribed to a gradual decrease in blood volume" (316).

Obviously, the default occurs in the cardiovascular apparatus. It is therefore appropriate to continue the story so well begun by Gregersen and to analyze the hemodynamic mechanisms that seem to be involved in transforming a state of impending shock to an irreversible form.

The only certain way to determine the existence of irreversibility is to test the cardiovascular response to infusions of blood or plasma substantially equal in volume to the fluid loss (138, 154). If the restoration of normal arterial pressure and contours of central pressure pulses is sustained a state of impending or reversible shock exists, but if a progressive decline of arterial pressures and deterioration in the contours of central pressure pulses supervene

in the succeeding two to six hours, a state of shock irreversible by transfusion is present. The hope must not be abandoned that it may prove reversible by other means. However, the transition from an impending to an irreversible state after massive hemorrhage, and presumably any other fluid loss, can often be inferred when a low arterial pressure is not prevented from falling further by small blood transfusions and when hematocrit readings start to increase. A probably fatal outcome following restoration of blood volume is signaled by actual hemoconcentration, a rapid heart rate in relation to control rate, and passage of bloody fecal material (155). Evidences of cardiac dilation are also ominous (156, 157). The progressive decline of arterial pressure which follows restoration of control blood volumes in animals in an irreversible state is initially due to recurrence of reduced cardiac output (158). While myocardial depression is partly responsible (140, 156), progressive reduction in venous return is of paramount importance (147).

Default of a venopressor mechanism is still considered important by some investigators. This is inferred from measurements of intramuscular pressure in patients (159, 160). Previous reports that intramuscular tension is increased by bleeding and restored to normal after reinfusion of heparinized blood have been confirmed (161). However, release of tourniquets in deeply anesthetized dogs, application of pressure to a limb, trauma, and gunshot injuries reduce intramuscular pressure (161). The significance of such measurements remains highly debatable, for (a) direct measurements of muscle tension by isometric levers have failed to detect changes in muscle tone in hemorrhagic shock (162). (b) abolition of all muscular tone by curare preparations does not lead to shock (163), and (c) neither nikethamide, which increases muscular tonus nor curare, which induces muscular paralysis, affect the bleeding volumes of mice in burn shock (164).

Henderson's thesis that hypotonia is concerned in shock and that the former can be estimated by measurements of intramuscular pressure is also rendered unlikely by other experimental evidence. The modified Riml experiments of Ralston *et al.* (165) indicate that abolition of muscle tonus by deep anesthesia, intocostarin, or death somewhat reduces the average quantity of blood that can be drained from the central veins after abrupt clamping of the aorta and pulmonary artery. As the authors point out, the rates of collection and volumes of blood yielded in such experi-

ments depend on the level of the collecting reservoir and, in our experience, on a variable quantity captured and not easily drained from the right ventricle. Therefore, too much significance cannot be attached to the average differences obtained. On the other hand, it has been found that, while abrupt, prolonged vagal standstill of the heart is not followed by an appreciable increase in central venous pressure after large losses of blood, it does increase substantially during the circulatory failure which follows reinfusion of all withdrawn blood (140). The nature of the defect in peripheral mechanisms has been elucidated by two approaches: (a) hemodynamic studies, and (b) direct observations of blood vessels; both by improved techniques.

*Hemodynamic studies.*—Changes in total peripheral resistance (T.P.R.) calculated from measurements of cardiac output per min. and mean arterial pressures are extremely variable during impending and irreversible shock in dogs (158, 166). This is also true after hemorrhage and in various clinical types of shock (129, 167). Following massive hemorrhages in dogs, increase in total peripheral resistance does not always occur and is never maximal. Its role in sustaining arterial pressure has probably been overestimated. However, the general trend is toward a slight or a marked increase during impending shock and toward a definite decrease during transition to the irreversible state. Substantial reinfusions restore or increase total peripheral resistance in relation to control levels, but in terminal stages total peripheral resistance decreases again and is chiefly responsible for the ultimate decline of arterial pressures to low levels (158).

Changes in total peripheral resistance, of course, do not reflect proportional changes in vascular resistance due to vasomotor action (p. 269), especially when changes in viscosity and large changes in arterial pressure occur (cf. p. 270). In states of shock accompanied by marked increase in viscosity development of irreversibility is favored because shocked dogs cannot respond by decrease in vascular resistance, as do normal dogs (98). Since changes in total peripheral resistance also give no information regarding flow and resistance in various territories, the latter have again been studied with improved methods. During impending and irreversible shock increased resistance has been demonstrated in the limbs (42, 91, 173), the kidney (69, 71, 73, 168, 169), the liver (84), and the spleen (170). The chief function of the splenic con-

traction appears to be to recruit blood for the general circulation (170, 171). However, resistance is reduced in territories supplied by the mesenteric arteries during development of the irreversible state (172), and it is reduced in the coronary vessels throughout the course of impending and irreversible hemorrhagic shock (174). The inference is that the reduction in total peripheral resistance during irreversible states is dominantly due to reduced splanchnic and coronary resistance. The latter is supported by reports of morphological changes in myocardial capillaries (175, 176) and metabolic alterations of the myocardium (177). The former is supported by frequent occurrence of intense congestion, edema, and hemorrhage in the mucosa of the upper intestines of dogs, and by direct observations of blood vessels now to be analyzed.

*Direct observations of blood vessels.*—Direct observations of blood vessels by techniques devised by Clark and Clark, Knisely, Page and Abell, and by Chambers and Zweifach have revealed reduction in size of small arteries (0.4 to 1.2 mm.), and arterioles (24 to 65 $\mu$ ) in the mesentery of cats following release of limb tourniquets (178) and after burns (179), in the mesentery of dogs after hemorrhage exceeding 2 to 2.5 per cent of body weight (180), and in normal and denervated ears of rabbits following intestinal manipulation (181). Capillary ischemia is reported by many of these observers. Increase in size of minute vessels, slowing of flow, and capillary engorgement seem to occur as terminal events only. However, intestinal manipulation and release of tourniquets in rats are said to cause almost immediate opening of new capillaries, congestion, and stasis in the mesentery, and opening of functional sinusoids, without congestion, in the liver (182).

Such general descriptions of changes in static sizes of small vessels must be accepted cautiously in drawing conclusions regarding vasomotor changes, for their diameters decrease passively with reduction of blood pressure. Furthermore, it is not always clear whether descriptions apply to states of impending or irreversible shock. The utilization by Chambers & Zweifach (93) of periodic active changes (vasomotion) as a criterion of vasomotor activity is a distinct advance. According to their studies, constriction of minute skin vessels precedes that of visceral vessels and is induced by smaller losses of blood (183). In the mesoappendix of rats this is accompanied by accentuated vasomotion of metarterioles and precapillary sphincters, and hyperreactivity to minute doses of

epinephrine. As a result of such constriction, less blood enters the true capillaries and accounts for their ischemic appearance, but larger volumes are shunted into venules by small direct arteriovenous channels. When the venules fill too much, blood flows back into true capillaries and this causes stagnation. Similar changes were seen after traumatization in the Noble-Collip drum (184). In tourniquet shock (185), several stages occurred in sequence (in rat mesoappendix): (a) A dilatation stage following release, characterized by enlargement of arterioles, persistence of vasomotion and hyperemia. (b) A hyperreactive stage (described above), the duration of which is related inversely to the time of tourniquet application. (c) A hyporeactive stage during development of low blood pressure characterized by enlargement of arterioles and venules, cessation of active vasomotion, diminution or abolition of response to epinephrine, capillary stagnation, and slower flow. Later, similar observations on the omentum of dogs were correlated with hemodynamic changes in graded hemorrhage and reinfusion experiments in dogs (186). According to these studies reinfusion causes only partial and temporary recovery in arteriolar constriction and metarteriolar vasomotion. Hence, while the rates of capillary and venular flow increase temporarily, the capillaries are not emptied of their pooled blood; on the contrary, the pooling is intensified when the integrating vasomotor mechanism fails again. Venous return decreases progressively because the capillary bed becomes an inert network. The conclusion is drawn that in dogs, cats, and rabbits (187) reduction in venous return, cardiac output, and arterial pressure in shock are due to loss of fluid and sequestration of blood in capillaries and venules due to operation of a hyporeactive factor. The further conclusion that in hemorrhagic shock this is in some manner linked with liver damage is supported by the observations that maintenance of an adequate blood flow through the liver by perfusion from another dog seems to protect an animal from development of irreversible hemorrhagic shock (188, 189).

While paralysis of vasomotor action on the arteriolar side of mesenteric capillaries seems to be an important factor in the development of capillary stagnation, such pooling of blood must also be aided considerably by the portal congestion which develops through increase in hepatic resistance (84) and failure of the spleen to absorb this blood by relaxing (170). Indeed, experiments in progress in this laboratory indicate that the congested appearance

of the upper intestinal mucosa observed after hemorrhagic shock can also be produced by partial obstruction of the portal vein.

In summary, the progressive reduction in venous return which develops during states of normovolemia (i.e., following large transfusions) is probably not due to any single but to multiple vascular changes, among these (a) loss of vasomotion in certain visceral territories, (b) passive portal congestion due to persistent increase in hepatic and splenic resistance, (c) marked reduction in renal flow due to progressive increase in renal resistance, etc.

The extensive work of Knisely and his associates has suggested that in malaria the peripheral circulation is slowed by alterations in the consistency of blood produced by adhesion of white corpuscles, formation of a glassy precipitate, and to the clumping of red cells, in short, by the formation of sludge. In an extensive paper (190) the conclusion is reached that similar alterations occur in traumatic shock. A careful reading of this report indicates that precipitation and agglutination effects were noted chiefly in regions in proximity to injuries, e.g., in experimental crushing of the monkey's omentum. Their inference that this sludge passes into the general circulation and can be demonstrated in remote vessels such as those of the conjunctiva was not documented by valid evidence, and requires confirmation.

*Concerning other causative factors in traumatic shock.*—While the preponderance of evidence favors the view that reduction of blood volume is the primary factor in shock, this is not generally conceded. The circulatory failure which occurs after large transfusions following prolonged severe hemorrhagic hypotension is not characterized by any marked reduction in blood volume (191). Shock also appears to follow release of tourniquets under conditions in which local fluid loss is less extensive or is compensated by reabsorption from other regions (192 to 196). Apparently, tourniquets applied with different techniques evoke varying grades of edema. As a rule, considerable swelling occurs, but after heating of the leg tourniquet compression causes little loss of fluid, owing to thrombosis of major arteries (197). Furthermore, casting of the hind legs and abdomen after complete block of venous flow may delay a fatal outcome, but does not prevent it (198). Others have measured or inferred that reductions in blood volume do not offer an adequate explanation for all types of shock (159, 160, 199).

Failure to demonstrate that the local loss of fluid in trauma



does not equal the loss of blood required to produce shock does not necessarily warrant the conclusion that toxic agents are concerned. Attempts have been made to demonstrate this, among other ways, by cross-circulation experiments. (For references to previous literature see 208, 316.) Recent efforts to demonstrate toxic factors in this way have also not proved uniformly decisive, but suggestive (194, 200, 201). The nature of the toxic agent remains enigmatic. According to Cicardo (202), potassium is released after removal of tourniquets, and electrocardiograms taken at this time resemble those due to potassium injections (203). However, the bulk of evidence continues to indicate that marked increase in blood potassium is solely a terminal event (201). Furthermore, increase in blood potassium has been noted after spinal cord section at the eighth cervical segment which does not eventuate in shock (204, 205), and the toxic agent in fluid drained from the leg after release of tourniquets is not diffusible (210). While bacterial controls indicate that traumatic shock can be produced in the absence of infection (197), the comprehensive studies of Aub and co-workers (206 to 211) indicate that bacterial toxemia can play an important role (211). This may explain clinical types of circulatory failure in infectious diseases (212), and must be further checked as a possible factor in hemorrhagic shock in which heparinized blood is reinjected. It has been suggested that the spread of edema to adjacent areas which have not been traumatized may be due to vascular dilation or to capillary injury produced by absorption of toxic material. Such assumptions are unnecessary; the spread of fluid is fully explained on a physical-anatomic basis (213). The apparent immunity developed by animals against trauma is local in origin (214).

Circumstantial evidence that afferent nervous factors play a role in traumatic shock has again been brought forward (215), but clear-cut experiments of Phemister and co-workers (216, 217) continue to negate such claims. Cerebral ischemia of a degree sufficient to decrease carotid back pressure to 30 mm. Hg leads to increased peripheral resistance. If this is continued for a sufficient period, it is followed by decreasing peripheral resistance and circulatory failure (96). While this strongly suggests that efferent vasomotor impulses were concerned in these experiments it does not necessarily prove that similar mechanisms are involved in other forms of shock. The existence of hypertension previous to bleeding does not

seem to influence the development of shock or life expectancy (218).

*Therapy.*—The volume of blood lost in operations (219, 220) and in battle casualties (221, 222) is unexpectedly large and the importance of early replacement has been stressed. The clinical use of barbiturates has been discussed (223, 224). All anesthetics act unfavorably on the heart and circulation after shock has developed (225). The choice depends on aims it is hoped to accomplish. Morphine is considered superior to barbital for animal experiments by some investigators (226, 227), but the latter in biologically standardized doses does not seem to affect the peripheral circulation (182, 228), and renders dogs (229) and guinea pigs (230) more resistant to hemorrhage. However, large doses of pentobarbital are said to produce vasodepression (231). A favorable aspect of barbiturates is that they reduce fluid loss in burns (232) and capillary permeability generally (233), a property not shared by morphine.

No significant advance can be recorded in drug therapy. Pressor drugs and cardiac stimulants such as tuamine have proved ineffective (154). Liver extracts which exhibit antishock action in burns have no such actions in inflammatory, tourniquet, and hemorrhagic shock (234). Oxygen administration appears advisable to prevent respiratory depression during operations on patients in shock (235). Intravenous oxygen therapy has again been tried with optimistic interpretations (236). Increase of histamine in blood occurs after burns and may be concerned in the etiology of gastric ulcers (237). Traumatic shock in dogs which is not influenced by plasma transfusions is said to be benefited by cholinesterase; the *modus operandi* remains unknown (238). Pretreatment of guinea pigs with large doses of vitamin C prevents development of shock by a standard type of bleeding (230), but curative value has not been demonstrated.

The value of saline solutions apparently depends on many circumstances, among them, the time of administration (239), and the use of adequate volumes (240 to 242); but avoidance of pulmonary edema and ascites is important (154, 188). Fluid priming and alkalization may delay transition to irreversible states, but does not reduce mortality rates in hemorrhagic shock (154, 188, 229, 241, 243). Administration of sodium chloride is apparently of greater benefit in circulatory failure following salt depletion than

in that due primarily to dehydration (244, 245). However, the evidence that salt depletion eventuates in vascular collapse (244) is not very convincing (great increase in T.P.R. with terminal arterial pressure ranges of 78 to 142 mm. Hg.). Evidence continues to be presented that refrigeration of traumatized parts exerts a favorable action (242, 246, 247) and that maintenance of low environmental temperatures extends the survival time. Various ranges have been considered optimal, e.g., about 15 to 20°C. (248); about 16°C., or below (249); about 18 to 20°C. (250); about 9 to 14°C. (251). However, extreme reduction of body temperature by such means is deleterious.

*Hemorrhage and shocklike states.*—The cardiovascular changes following removal of 300 to 1,000 cc. of blood by venesection and the effects of pooling of blood in the extremities have been studied further in man. Low arterial pressures and a rapid pulse are not generally present in recumbent subjects, but develop on arising (252). Cardiac output is not decreased until more than 500 cc. is withdrawn (252), despite the fact that right atrial pressure falls (129). The acute circulatory collapse which occasionally follows withdrawal of small volumes of blood is characterized by marked cardiac slowing and sudden reduction of arterial pressure. Atrial pressure increases, cardiac output remains unchanged, but total peripheral resistance decreases (129). Dilatation of blood vessels in skeletal muscles seems to occur (37). Similar reactions leading to syncope after venepuncture, distention of organs, stimulation of the carotid sinus, hyperventilation, etc., have again been studied (253–55). Individuals in whom bradycardia, local changes in cerebral blood flow, and vasodepression respectively, seemed to dominate were studied. Vasodepression, the most common factor may be due to psychogenic or reflexogenic dilatation of arterioles, or may result from physiologic or structural disorders which impede venous return. The most common causes for this type of circulatory failure are hemorrhage, fever, strenuous exercise, dehydration, prolonged recumbency, early pregnancy, and postinfection states (255). The condition is apparently related to orthostatic circulatory failure or gravity shock which has been extensively studied (256). Further studies by Mayerson (257) suggest that the orthostatic circulatory failure is essentially equivalent to impending shock (cf. p. 278), and can be made irreversible by withdrawal of relatively small quantities of blood (30 to 50 cc. in dogs). Irreversi-

bility can be prevented by infusions of either blood or saline solution during the upright period.

The reader should compare this review on shock with several others which summarize current opinions developed by different groups of investigators in the light of their own work (188, 258 to 260, 318). In concluding this topic, the writer cannot refrain from stating that an unwarranted repetition of the same experimental material in different journals is noticeable in the recent literature on shock.

#### HYPERTENSION

Recent developments in the hypertension problem have been covered in the monographs of Goldring & Chasis (314), and of Page & Corcoran (323), and in Goldblatt's review (313). Phases of the subject were also discussed by active workers in a recent conference (325). The relation of hypertension to the renal pressor mechanism is discussed in the chapter on the kidney in this volume. A number of basic problems remain unsolved and continue to be the chief subjects of investigation.

The commonly accepted renin-angiotonin theory is reviewed in detail by Goldblatt (313), and its applicability to human hypertension is analyzed. The view is supported by several clinical reports: several cases of hypertension were accompanied by obstructive lesions in one or both renal arteries (261), and constriction of the renal artery for twelve minutes resulted in release of renin into renal venous blood (262).

While accepting a renal origin of hypertension, Ogden and his group (325, 263) have proposed that the renal pressor system is a homeostatic mechanism regulating the circulation and that it produces disease when stimulated too long. Later they suggested that a fundamental change of mechanism occurs in the later stages of experimental renal hypertension, the renal pressor system being supplanted by a neurogenic one (264). This is based on their experimental findings that removal of the restricted kidney, or administration of pentobarbital, yohimbine, and F-883 in hypertensive rats, cause different responses, depending on the duration of hypertension (264, 265, 266).

Grollman (267, 325) has opposed the theory that the chronic experimental hypertensive state is due to a renal pressor mechanism induced by an injury or ischemia of the kidney tissue. Rather, he believes that it is caused by a deficiency of renal function. Con-

striction of the kidney or renal artery, the most widely used methods for the experimental production of hypertension, is thought to interfere with the normal function of the kidney, which is the maintenance of a normal blood pressure. The absence of an unknown renal humoral agent is considered to be responsible for hypertension. However, the formation of a pressor substance in acute experiments is not denied. These concepts have been used as a rationale of certain methods of treatment (268).

The relationship of arteriosclerosis to hypertension has been critically reviewed by Hueper (269) and commented on editorially (270). The vasopressor principle, released as the result of renal ischemia, is thought to play some role in the production of this syndrome. No definite decision can be made as to whether hypertension or arteriosclerosis is primary. It is interesting that in experimental renal hypertension, Goldblatt has failed to find arteriosclerotic changes in the benign phase, and often no morphological changes at all, while in the malignant phase necrotic, but not arteriosclerotic, alterations could be seen (325).

The theory that the kidney is the primary factor in the production of clinical hypertension has been challenged by some investigators who consider the renal origin unproved and who regard the kidney as "the victim rather than the culprit" (271, 325). Goldring & Chasis (314) have elaborated on this in their monograph. According to them, a humoral mechanism of unknown, but not renal, origin is probably involved. Intrarenal vascular disease, a common pathological finding associated with hypertension, is to them secondary to the hypertensive state. This conclusion has, however, not gone unchallenged (313).

The results following Smithwick's lumbodorsal sympathectomy (272, 273), while primarily to be evaluated in terms of their clinical success, have been taken to hold important implications for the pathogenesis of hypertension and provide a clue as to its etiology, or at least its development. The role of this procedure in clinical medicine has been discussed editorially (274). The extent to which this method of treatment may substantiate Ogden's theory (264) has not been clarified. Production of a prompt and sustained hypertension by the unilateral ligation of periadrenal blood vessels and tissues in dogs has been claimed (275). It has so far, however, not been confirmed.

Many suspicions exist that nervous factors are of importance in the genesis of hypertension, but crucial experimental evidence

has been difficult to obtain. Several observers have again reported success in producing experimental hypertension by complete section of the aortic depressor and carotid sinus nerves (218, 276). However, the hemodynamics of such neurogenic hypertension differs materially from that observed in experimental renal hypertension. Total peripheral resistance is not changed or increases only slightly, while cardiac output is greatly increased due to cardiac acceleration. Renal arteriolar constriction occurs with normal blood flow through the kidney in most dogs, and the blood flow through the forelegs increases due to decreased resistance (277). Weaned rats exposed daily for ten to fifteen minutes to a noisy air blast develop hypertension as they age (278). The possibility that nervous influences might produce hypertension by reducing renal blood flow was tested by stimulating the renal arterial plexus for many days. Hypertension developed during the period of stimulation but did not continue after cessation (279). These investigators point out frankly that it remains to be demonstrated that comparable nervous effects are continuously operating in human hypertension or that an irreversible hypertension can be established after more prolonged stimulation.

Hemodynamic changes in hypertension have been studied further. Changes in blood flow in the skin before and after performance of the Smithwick operation have already been mentioned (47, 48). However, a greater than normal blood flow through the limbs, considered, without proof, as differential vasotonic reactions of surface and splanchnic vessels, has been reported (280). Hemodynamic alterations were compared in normotensive and hypertensive subjects during pyrogenic reactions, special attention being paid to cardiac output, stroke volume, and peripheral resistance. Hypertensives showed poor circulatory adjustment to postural changes under the stated conditions (281). The use of renal function tests to distinguish neurogenic from humoral vasoconstriction has been discussed (282).

The range of blood pressure in human hypertension has been considered in relation to its changes with the duration of the disease and the possible differentiation of humoral and vasomotor components (283). Systolic and diastolic pressure levels in transient hypertension have been treated statistically for their prognostic significance in the later development of persistent hypertension (284). Any slight elevation is considered important, even without increase in diastolic pressure. There appears to be correlation be-

tween the rise in blood pressure and the severity of any later lesions. Blood pressure alterations of hypertensive patients at rest and after sedation or anesthesia were found too variable to provide a basis for prognosis or etiologic assumptions, without their integration into the total clinical picture (285).

The relationship of the hypophysis to hypertension has been investigated. It was found that the posterior lobe is not necessary either for the initiation or the maintenance of experimental renal hypertension (286). Hypophysectomy, however, causes a fall of blood pressure in hypertensive rats. A return to prehypophysectomy levels was observed on administration of adrenocorticotrophic hormone, indicating that the adrenal cortex is probably involved in a renal pressor mechanism (287).

#### BUBBLE FORMATION

Harvey (319) has reviewed and discussed the fundamental problems concerned, and Gersh (312) has brought the physiological aspects of compression sickness up-to-date. The medical importance of recent work has received editorial comment (288) and its application to aviation medicine has been stated (289). The recent contributions will therefore be indicated with great brevity.

Carbon dioxide has been found to play an important part in the initiation and early growth of bubbles, although nitrogen gas is principally responsible for their later growth and maintenance (290). Several factors concerned in the effect of exercise to increase the rate and degree of bubble formation have been studied (291, 292). Muscular immobilization by pentobarbital in decompressed rabbits favored survival (293). Preoxygenation has been found of value in avoiding or reducing bubble formation, probably due to the removal of nitrogen (293-295). The comparative values of oxygen, helium-oxygen, argon-oxygen, and air were determined showing that oxygen alone was most effective (296). Exercise before decompression was found to increase the rate of removal of nitrogen from the tissues, and the mechanism was elucidated (295, 297). Some additional factors in the origin of bubbles have been studied (298). Gas bubbles on decompression have been observed in the bone marrow, lungs, and spleen (299). They were also found in pial vessels, where they were carried from other parts of the body (300). A few bubbles could be observed in the coronary arteries, but gas emboli in the central nervous system were the usual cause of death (301).



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DEPARTMENT OF PHYSIOLOGY  
WESTERN RESERVE UNIVERSITY MEDICAL SCHOOL  
CLEVELAND, OHIO

# HEART<sup>1</sup>

BY S. RODBARD AND L. N. KATZ

Cardiovascular Department, Research Institute,  
Michael Reese Hospital, Chicago, Illinois

## EMBRYOLOGY, ANATOMY, AND PATHOLOGY

The process of cardiogenesis in the sheep was traced from the first stages to the formation of an unpaired heart rudiment (1). Separation of the two halves of the heart anlage of *Amblystoma punctatum* at stages 14 and 15 caused each half to develop into a more or less normal heart. The situs of the left heart was invariably normal while the right heart showed situs inversus in most cases with a clear-cut relationship of parts. It was concluded that normality of heart structure during development depends to a large extent upon its freedom from mechanical pressures by the graft (2).

An extensive comparison of growth differentiation, activity and action currents of heart and skeletal muscle was made in hanging drop tissue cultures of chick embryos. In both types of muscle, activity preceded the appearance of myofibrillae and cross striation. When isolated fragments of atrium and ventricle were cultivated side by side, each contracted at first with its own rhythm, but later, when fusion between them was accomplished, they formed a single contracting unit beating at the frequency of the atrium (3).

Study of the postnatal growth of the heart of the albino rat showed that at birth the walls of the left and right ventricle were of approximately the same thickness. This ratio changed immediately after birth so that on the fifth day it was 2 to 1, on the tenth day 3 to 1. From this time on growth of the thickness of the two ventricles was nearly equal so that at maturity, 150 days, the ratio was 3.4 to 1 (4). Faller (5) has made an extensive study of the human epicardium.

While the human atria are normally supplied with six or more arteries, a case is described in which they are supplied by only two arteries (7). The old question of the normal vascularity of the cardiac valves is still being studied (8). It has been shown that normal heart valves of man and rabbit are largely nonvascular. In

<sup>1</sup> This review covers the period from July 1, 1945 to June 30, 1946.

diseased valves, blood vessels form as part of the inflammatory response. Particulate damage to the tricuspid valves of the rabbit induces a new formation of blood vessels. The general receptivity of the cells in the normal and damaged tricuspid of the rabbit to Trypan Blue is described (9). Heart valves of many mammals are normally vascularized so that care must be exercised in the interpretation and comparison of results of experiments aimed at the production of endocarditis in different species (9, 10). Asymptomatic calcification of the mitral valve especially in the annulus fibrosus occurs in 10 per cent of unselected hearts. This is not the result of an inflammatory process (11).

The effective use of penicillin in the treatment of subacute bacterial endocarditis has provided opportunities to study the natural history of healing or healed endocarditis of this origin. It has been found that these patients may succumb to heart failure especially if there is an aortic regurgitation, apparently because this defect becomes aggravated (14, 15, 16). An excellent review of coronary occlusive disease has appeared emphasizing the presence of coronary disease in infants and children (17) and the subject of atherosclerosis has been resurveyed (18). In five hundred necropsied cases of sudden death, coronary atherosclerosis and its sequelae were found to be the principal cause of death (19). The technique of Schlesinger was used, in a study of the incidence of coronary arteriosclerosis and myocardial infarction, to determine the amount of collateral circulation present. Interarterial anastomoses were demonstrated in all hearts with pronounced arteriosclerotic narrowing of the coronaries (20). This confirms well established knowledge. It was shown that in the dog these collateral channels may quickly widen when an acute coronary closure is produced (21).

Robb & Kaylor (22) have shown that the conduction system of the guinea pig heart is easily demonstrated and differentiated from ordinary muscle fibers. The distribution of the right and left branches of the auriculo-ventricular bundle is similar to that seen in other animals. However, there are additional septal branches not seen in most mammalian hearts (22). In eighteen out of twenty human hearts the auriculo-ventricular bundle was bound beneath the pars membranacea septi under the septal cusp of the tricuspid valve. The right branch could be exposed in part in most of the hearts: in some, portions of it could be seen through the endocar-

dium, and in others, the entire length of the right branch stood out clearly without dissection. The left branch was difficult to expose and varied in its method of origin. The auriculo-ventricular node was dissected as a demonstrable entity in five hearts in each of which the nodal artery was found arising from the right coronary (23). These studies are pertinent in view of the recent trend in certain quarters to doubt the existence of the special connections between atria and ventricles in many species of mammals including man. A survey of the older and recent literature will show equally convincing reports of their existence.

Very complicated sensory nerve endings of two types and consisting of spirals that encircle the muscle fibers of the myocardium and some which are similar to the neuromuscular spindle receptors of the skeletal muscle have been observed in the myocardium of man and cats (24). The presence of numerous sensory fibers in the walls of the coronary vessels has been confirmed. In some cases, the sensory endings of the myocardium and those of the coronary vessels originate from the same fiber (24, 25). The role of these end organs in the pathogenesis of pain and in the control of the cardiovascular system merits careful consideration.

The recent advances in the corrective surgery of the defects in some forms of congenital heart disease has made this another form of therapeutically reversible heart disease (26). This concept pertains to patent ductus arteriosus (27) but will apply also to the correction of coarctation of the aorta (28). The value of the operation of Blalock & Taussig (29) for establishing an artificial "patent ductus arteriosus," while symptomatically often spectacular, should remain *sub judice*. The increased load on the heart may, in the long run, counterbalance the possible benefits.

#### IRRITABILITY, RHYTHMICITY, CONDUCTIVITY, AND TONUS

The frog heart continued to beat for two hours in an electrolyte-free medium (half isotonic sucrose). During this period it gave a graded response to induced shocks, resembling the action of striped muscle in this respect (30). When the excised frog heart was perfused with Ringer's solution of gradually rising temperature, alternating periods of activity and rest became apparent at 33°C. In the presence of atropine or physostigmine this critical temperature is raised to approximately 38°C. (31). Intermittent beating or arrest of the Straub-Fuehner frog heart preparation also resulted

from stimulation by means of a modulated high frequency condenser field. Prolonged exposure produced irreversible changes (32).

A gradual decline in heart rate with extreme anoxia was seen in the rat, falling from 310 beats per min. at 5280 ft. (ground level) to 222 beats at 40,000 feet simulated altitude (33). Rapid stimulation by induction shocks of the Ringer perfused frog heart was reported to cause a tetanic response. This response did not occur when additional calcium was added (34). This effect may have been due to perfusion with Ringer's solution in which the calcium was inadvertently precipitated out.

Magnesium and calcium decreased the rate and tonus of the isolated ventricle of *Helix*, an effect antagonized by potassium. Lower temperatures favored the magnesium effect, higher temperatures that of potassium (35).

At very high rates of beating of the whole auricle and of ventricular strips of turtle heart, atropine directly depressed conductivity, but when the rate was slow it had no constant effect on rhythmicity, contractility, tonus, absolute refractory period, or on conduction (38). Atropine and physostigmine apparently raised the initial temperature threshold for the cyclic activity pattern of the excised frog heart preparation (31). Atropine increased the atrioventricular nodal rate in three dogs in which the sinus node had been destroyed indicating that the vagus influences the auriculo-ventricular node (39). Barbiturates depress the activity of the excised frog heart (40, 41).

Premature systoles were produced in man by direct mechanical irritation of the left or right ventricle (42). Reflex inhibition of the sinus node, and sometimes of the auriculo-ventricular node, could be elicited by mechanical stimulation of the epicardium in the region of the sinus node (43).

The heart rate was unaffected by the development of motion sickness (44). In an apparently normal athlete, a prolonged P-R interval of 0.39 seconds was found (about double the normal value); this was halved by exercise or atropine and increased somewhat by acetylcholine (45). It is thus neurogenic and serves to show that apparently abnormal values for various cardiac properties may occur in the absence of disease of the heart.

In volunteers, cyanide caused a sinus pause of one to four seconds, concomitant with the onset of respiratory stimulation, and this was followed by slow irregular P waves for several seconds



with gradual acceleration beyond the control levels. The response was more marked in three men executed with cyanide (46). Decherd (47) has emphasized the role of varying rates of recovery of conductivity as a cause of the Wenckebach phenomenon in partial auriculo-ventricular block. An unusual case of complete auriculo-ventricular block following an explosion which shot a shell fragment into the heart of a soldier is reported but no post mortem was done (48). Lenègre (49) made an extensive microscopic study of a case of complete auriculo-ventricular block and found the lesion (vacuolar degeneration) in the left bundle branch system. In addition, proliferating and stenosing endarteritis of the blood vessels supplying the auriculo-ventricular bundle were present.

*Ventricular fibrillation.*—Clamping of a coronary artery was much more effective in producing ventricular fibrillation than generalized anoxia or hemorrhagic shock. The frequency of fibrillation in coronary occlusion affecting a local area and the rarity of fibrillation in conditions affecting all parts of the heart is consistent with the findings that fibrillation is prone to occur under conditions producing rapidly discharging ectopic foci, or that the production of an ischemic-nonischemic boundary is a source of the ectopic foci. Anoxic areas, being hypoactive are thus not likely to produce fibrillation (50). These conclusions are very important in clarifying the controversy over the mechanism of production of active ventricular ectopic rhythms, especially after certain types of anesthesia, or with adrenergic substances.

The mechanism whereby anesthetic agents produce active ventricular rhythms is under investigation. Earlier work is reviewed by Meek (51). While some authors (52) still hold that chloroform acts by induction of anoxic anoxia, other findings suggest that chlorinated hydrocarbons, among other effects, increase the amount of free acetylcholine (53) and sensitize the myocardium, possibly through central sympathetic stimulation (54). Demerol, an opiate, protects the heart from irregularities produced by cyclopropane (55). Protection from fibrillation by fagarine (56), adenosine triphosphate (57) and atabrine (58) has been reported.

The literature on auricular and ventricular fibrillation is reviewed and additional studies reported on the turtle (59). Neither ventricular fibrillation nor vagus standstill increase the potassium content of the blood plasma or cause any significant change in total blood phosphorus or total acid soluble phosphorus of plasma

(60). The use of alternating current as an antifibrillatory agent is still being reported upon. Ventricular fibrillation was abolished and regular rhythm was restored by an alternating current of 1200 to 4800 volts applied to the thorax, or 240 volts applied directly to the heart (61) in a short intense shock (62).

The significance of rapid heart action in causing the Adams-Stokes syndrome is re-emphasized by a case report showing this phenomenon as a result of the rare condition of paroxysmal ventricular fibrillation (63). This case demonstrated that insufficient blood supply to the head may be produced by ineffective rapid heart action as well as by extreme slowing or standstill.

On the basis of his extensive pharmacological studies, van Dongen (64) has concluded that the circus movement theory for fibrillation is unacceptable. Heterotopic rhythms were produced in cats and dogs by injection of epinephrine or barium chloride, or by a small intravenous dose of strophanthin followed by ephedrine. Quinidine derivatives, 933F and F1262, inhibited fibrillation while barbiturates, analeptics, and miscellaneous drugs did not. The same dose of each drug which inhibited fibrillation also prevented other heterotopic rhythms while those drugs which did not inhibit fibrillation also had no effect on other ectopic rhythms. Some of the more active drugs such as emetine, novocaine, and 933F were able to suppress fibrillation without acting on the refractory period or on the conduction time. Drugs which altered the refractory period and conduction time also inhibited fibrillation but with less effectiveness than those which prevented ectopic rhythms.

#### CONTRACTILITY, CARDIAC OUTPUT, AND CARDIODYNAMICS

A number of compounds have been studied in relation to their effect on cardiac contraction. Thus, it is reported that adenosine-triphosphate (57) and coumagine, a digitalis-like nitrogenous phenathrene (65), increase the amplitude of the isolated mammalian heart. Atropine has no effect on contractility or tonus of whole auricle or ventricular strips of the turtle heart (38), while barium chloride depresses contractility (36). Acetylcholine consistently decreases the height of contraction and diastolic length of the turtle ventricle and atropine blocks these effects. However, the action of epinephrine is irregular and inconstant (66). Procaine had no effect on the amplitude of contraction of the isolated rabbit heart, while monacaine reduced the amplitude by 50 per cent (67).

With increasing concentrations of ethyl alcohol the amplitude of contraction of the isolated turtle heart was reduced, the heart was progressively dilated, and when the concentration was greater than 5 per cent fibrillation occurred (68).

Barbiturates were found to slow the isolated frog heart and decrease its amplitude of contraction. Ortol and seconal caused the greatest depression; amytal, pentobarbital and neonal caused moderate; and phenobarbital, evipal, barbital and vinabarbital, the least depression (40, 41). Intravenous injections of acid also impaired the contractility of the heart (37).

An aortic pressure of 45 mm. Hg is critical for the continued activity of the rat heart-lung-head preparation. At lower pressures, the heart fails rapidly (69). The failure of venous pressure to change in animals in irreversible hemorrhagic shock is attributed to a depression of the myocardium, i.e., to a reduced reaction to equivalent venous filling pressures (70). In support of this concept is the suggestion of reversible cardiodynamic deterioration in exsanguination shock (71). An extraordinary cardiac reserve was reported in anemic Puerto Ricans living in rural communities (72).

A theoretical curve of cardiac ejection as calculated from the contour of the aortic pressure pulse shows that marked variations may occur due to factors such as aortic tone, even when pulse pressure and stroke volume are kept constant (73).

The partial vacuum produced in the thorax by the excess of arterial outflow over venous inflow during cardiac systole would produce a negative pressure of as much as 15 mm. Hg on the lungs, but collapse of the chest wall tends to reduce this degree of vacuum. This partial vacuum tends to assist venous return but it is not as important as negative intrapleural pressure (74).

It is suggested that the epicardium assists the parietal pericardium in limiting the extent of dilatation of the heart during diastole (5). Boyd has shown that intrapericardial pressure usually tends to approach closely the intravenous pressure in the vena cavae. After hemorrhage the effective venous pressure is diminished (75).

*Cardiac output.*—In the study of cardiac output two methods continue to be employed in man: the direct Fick with catheterization of the right atrium or ventricle, and the ballistocardiograph. These have been subject to critical analyses. The relationship between the cardiac ejection curve and the ballistocardiographic

forces generated show that the values derived must be treated as comparative rather than absolute data. The need for an elaborate empirical research program to evaluate ballistocardiographic patterns in terms of disease and prognosis is indicated (76). Such a program has been inaugurated by Starr who after years of study is beginning to report his empirical findings. He reports that abnormal forms of the ballistocardiograph occur in older age groups, that they occur after surgical operations and disappear after recovery. Abnormal forms usually in association with heart disease are seen after procedures which diminish the venous return or affect the strength of the myocardium (77). Respiratory variations in the ballistocardiogram are due to displacement of the heart by respiration (78), and these are apparently associated with minor changes in cardiac output (78, 79). Use of the ballistocardiograph in the dog showed a reduced output in shock. Agreement with the direct Fick is only fair but when cardiac output changes the agreement in a given animal is considerably more accurate (80).

Cournand has reported on results in 260 trials with the method of catheterization of the right atrium or ventricle, a very useful technique which he had developed with so much success. In the normal male, the average cardiac index was found to be about 3.2 liters per sq. m. of body surface with a range of plus or minus 1 liter (81, 82). In a critical study of some of the errors in the method of right heart catheterization Warren *et al.* concluded that while the catheter method for measuring cardiac output is a useful procedure, the errors, which are random rather than systematic, are sufficiently large so that the values in one set of determinations may not represent the actual cardiac output (83). Twenty-two per cent of the blood oxygen determinations varied as much as 2.3 volumes per cent while 78 per cent had a maximum variation of 0.4 volumes per cent (83).

Using the catheter technique in four patients with an atrial septal defect, the output of the right ventricle was found to be at least double that of the left ventricle (84). In two of these patients with uncomplicated atrial defect, the right ventricular systolic pressure was found to be within the normal range, 40 mm. Hg, while in the third, who had evidence of pulmonary arterial disease, it was 112 to 146.

Slight transient increases in cardiac output occurred in hypertensives when they were tilted. After sympathectomy an equiva-

lent but more lasting increase was observed (85). Cardiac output was increased about two-thirds in anxious subjects with increased basal metabolic rates, and it was decreased somewhat by tilting the subjects to 70° (82). It is difficult to see how changes in cardiac output due to anxiety can be eliminated completely in catheterization technique.

Cardiac output increased during the reaction to intravenous injection of pyrogenic inulin (86) though it was variable in other febrile episodes (87). Active hyperventilation produced a mild increase in blood pressure but did not affect the cardiac output in young men. Passive hyperventilation produced a similar effect on the blood pressure but reduced the cardiac output by 10 per cent (88). Cardiac output was reduced during experimental pressure breathing (89). Infusion of gelatin solution produced a marked transient rise in cardiac output which was dissipated in twenty-four hours (90).

The cardiac output of the unanesthetized dog using the direct Fick technique was found to be 1.2 to 2.9 liters per minute, or 2.3 to 7.2 liters per minute per square meter of body surface (91). In experimental neurogenic hypertension, heart rate and cardiac output was increased (92). The effect of pulmonary embolism on the cardiac output as well as on other dynamic factors has been reviewed (93).

*Heart failure.*—A surprising report has appeared that the cardiac output (Fick method) falls to half of normal in congestive heart failure (94). Together with para-aminohippuric acid clearances which indicate that renal blood flow is one-fifth normal, this is interpreted to indicate that the edema is a result of forward, rather than the backward, failure. The importance of these observations is that much published data must be reanalyzed to determine wherein lies the contradiction between this report and earlier work which has been relatively consistent that cardiac output is not necessarily altered in cardiac failure.

In heart failure, mural thrombi and thromboses of the Thebesian veins may occur (6). Adequate heat elimination demands an adequate skin circulation and the latter places a significant strain on the heart, especially when heat loss by sweating is impaired. During congestive heart failure a minimum demand for skin circulation should be placed upon the heart (95). Patients with heart failure frequently show slightly subnormal albumin and slightly

increased globulin values; these return to normal levels after the dissipation of the edema (96).

The treatment of cardiac edema by means of a high fluid intake as promulgated recently by Schemm (97) has apparently proved to be of value. In a recent report on 402 patients, intakes of 3 to 5.7 liters per day permitted proper regulation of sodium in edematous patients and were beneficial (97). The usefulness of oral mercupurin in treating cardiac edema is re-emphasized (98). Perera (99) has presented evidence to support the concept of plethora in congestive failure by showing that the increase in plasma volume is associated with right-sided failure. Landis (100) has reported some beautiful studies on the relation of cardiac "competence" to the central venous pressure. Exercise and hyperpnea were found to reduce the venous pressure in the normal dog. Reduction of cardiac competence, as after ligation of the coronary arteries or the production of auricular fibrillation, reduced the ability of the heart to maintain a low venous pressure during exertion. When the embarrassment was extracardiac, as in cardiac tamponade or as a result of plethora, exercise reduced the venous pressure. It was concluded that the plethora of congestive heart failure is a compensatory reaction to many episodes of reduced effective blood volume occurring in the course of repeated transeint elevations of the venous pressure during muscular activity whenever venous return exceeds cardiac competence.

#### ELECTRICAL MANIFESTATIONS

Several new textbooks or revised editions of earlier texts on electrocardiography have appeared (101 to 105). The peculiarities of the electrocardiogram in several clinical states such as in acute rheumatic fever (106), epidemic parotitis (12), scrub fever (107), pneumonia (108, 109), glomerulonephritis (110), heat stroke (111), infectious mononucleosis (112), cardiac tamponade (113), pregnancy (114), anemia (115), uremia with pericarditis (116), and experimental starvation (117) have been reported. The subject of the short P-R-long QRS combination (the Wolff-Parkinson-White syndrome) continues to receive attention (118 to 123). The electrocardiogram in both clinical and experimental shock has been described (125, 126, 127).

With acidification of the blood with either hydrochloride or lactic acid, T and R amplitude progressively increased. S-T depres-

sion progressed rapidly in the few seconds before death (37). Emetine diminished the P-R interval by 0.03 second and tended to invert the T wave in man (128).

When maximal gravitational force on the centrifuge was maintained for ten to twenty seconds, the maximum heart rate was maintained constant. With the reduction in  $g$  in short runs there was a delay of two to five seconds before the heart rate suddenly fell to below its initial level. When subjects were explosively decompressed, bradycardia developed but there was no significant alteration in the electrocardiographic pattern (130). This is probably similar to the slowing of the heart seen during the Valsalva experiment due to other causes.

Several reports appeared on cardiac arrhythmias (131, 132) including paroxysmal tachycardia (13, 133 to 136). The persistence of electrocardiographic changes after the rapid heart action is over is again stressed (136, 137). Electric shock therapy is followed by sinus tachycardia and P and T changes. The QRS is unaffected (138).

Results of measurement of the area of the P wave in normals, in chronic mitral disease and in chronic pulmonary disease have been reported (139) and an extensive but not very significant series of reports has appeared correlating certain phases of the electrical heart cycle with variations in heart rate (140).

Electrocardiograms have been taken directly from the human heart by introducing an exploring electrode during pneumolysis. The voltage of the electrocardiogram on the heart was increased six times over that when the electrode was on the surface of the chest. The lead from the left ventricle was similar to that obtained from the left arm and the lead from the right ventricle was similar to that of the right arm (141).

The method for the construction of a vectorcardiograph is discussed at length (142). The stereogram (the three dimensional cardiovectorgram) may vary according to the location of the electrodes on the body, the most favorable condition being with two horizontal leads at waist level, and one vertical lead at the right side of the back (143). A new method for obtaining a sagittal electrocardiogram is described which is supposed to provide data for calculation of the vector quantity of the total electromotive force of systole (144). It is difficult to see how this can be true since it is contrary to all practical and theoretical conceptions.



The effect of direct current on the various components of the electrocardiogram has been reported by Rosenblueth (145) who insists that the true monophasic action curve is composed of a number of components. In view of the generally accepted concept this interpretation is unlikely and his results are most likely due to artefacts. Ventricular premature systoles appearing spontaneously after coronary occlusion showed configurations indicating that they originated in the damaged area. Thus, S-T<sub>1</sub> and S-T<sub>3</sub> were found to be depressed in injury to the right ventricle and elevated in injury to the left. In injury to the anterior wall, S-T<sub>1</sub> was elevated, and S-T<sub>3</sub> depressed while in injury to the posterior wall, S-T<sub>1</sub> was depressed and S-T<sub>3</sub> was elevated (146). The configuration of extrasystoles produced by stimulation of the endo-or epicardium of the ventricles is described (147). The electrocardiographic changes in a case in which the myocardial necrosis was more extensive or intense at the endocardial surface and at the apex than at the epicardial surface of the left ventricle was explained in accordance with the concepts of Wilson's school. The injury effects are the inverse of those that occur in acute diffuse pericarditis (148).

In a thoughtful article Eyster *et al.* (149) have again considered the source of the injury potential of the turtle heart and concluded that it is derived solely from the region of injury or the immediately contiguous tissue. The electrode on unimpaired tissue contributes to the recorded curve only by the addition of action potential components arising from the region of the electrode or from normal heart tissue intervening between the two electrodes.

No practical distinction can be made between an epicardial premature systole and its endocardial component as far as the appearance of the distant leads are concerned (150). This cannot be used, however, to contradict the early work of Lewis (151) that the initial part of the deflection in a lead consisting of one on the heart surface and the other on a distant body site is different for epicardial and endocardial surfaces. It therefore does not upset the ideas of "local distributed potentials" in the genesis of the electrocardiogram. This is also true of the report of Pruitt *et al.* (152) who found that lesions in the deeper layers of the myocardium have relatively little influence on the electrocardiograms compared with the effect of surface lesions.

The concept (153) of selective distribution of the electrocardio-

graphic patterns in the body is not substantiated *in toto* since it is reported (154) that the distributions of patterns  $C_1$  and  $C_6$  in leads do not always follow the pattern reported by Wolferth.

The distribution of net QRS and QRST potentials on the surface of the body were determined by Ashman who suggests that the use of Wilson's common terminal as a zero reference point introduces no very large errors (155).

#### OTHER MANIFESTATIONS OF CARDIAC ACTIVITY

A comparison of the pneumogram with the phlebogram showed the latter to be of more value in diagnosis of cardiac disorders (156). The circulation time in acute myocardial infarction is slowed by 3 to 24 seconds, and on recovery it returns to normal (157). It is confirmed that when the increased carotid pulse exhibits vibration at the height of the systolic excursion, called by the author "carotid shudder," which signifies that aortic incompetence and aortic stenosis coexist (158). The recognition and clinical significance of auricular heart sounds are discussed (159). On the basis of clinical studies it is assumed that the systolic sound in so-called systolic gallop rhythm originates in the pulmonary artery due to pulmonary stenosis (160).

Y. Henderson's work to the effect that there is a relationship between pulse rate, cardiac stroke volume and the oxygen requirements of the body during rest and exercise has been confirmed, and it is again suggested that one or more of these functions are useful as indices of fitness (161). With training, smaller increases in heart rate occur during exercise (162, 163). An analysis of the circulatory responses of the champion runners Gundar Hagg and Arne Anderson shows nothing out of the ordinary (164). After a three-week period of bed rest, the average pulse rate in normal subjects walking 3.5 miles on a 10 per cent grade was increased from 123 to 169. A reconditioning program brought the rate down to 145 in one week, and back to control values in four to six weeks (165).

#### NERVOUS CONTROL

Few alterations in the intra- and extracardiac sympathetic ganglia were seen in a study of fifteen pathological human hearts (166). Simultaneous electrical stimulation of both vagus nerves in the dog with heart standstill does not increase plasma potassium

content in the blood drawn from the left ventricle (60). Why the investigators did not collect right ventricular cavity or coronary sinus blood for this study is difficult to understand.

Intracisternal injections of potassium phosphate in dogs were followed by the production of ectopic beats, tachycardia, and bizarre complexes in vagotomized animals (167). Persistent ventricular bigeminal rhythm in women is apparently related to some emotional factor (131). Emotional and mental strain associated with the war, and excessive use of tobacco are probably factors in the production of paroxysmal auricular fibrillation (168). Undoubtedly the greatly increased use of coffee may also play such a role in sensitive individuals. Heightened sympathetic activity rather than body build, a change in cardiac rotation, or diminished coronary flow is chiefly responsible for the  $T_1$  and  $T_2$  changes in neurocirculatory asthenia (169).

There has been a tremendous activity among organic chemists and pharmacologists in the synthesizing and testing of spasmolytic and atropine-like drugs (170 to 175). Comparison of the actions of these compounds will undoubtedly be of value in unraveling the mode of action of parasympathetic drugs.

It is of extreme interest that three different laboratories have almost simultaneously reported that acetylcholine appears to produce compounds with epinephrine-like action. Thus, large doses of acetylcholine or nicotine cause increase in rate and amplitude with coronary dilatation in the isolated atropinized heart of several mammals. The perfusate from such hearts exerts a positive inotropic action on the hypodynamic frog heart and relaxes small intestine, attributable to presence of an epinephrine-like substance in the perfusate (176). Haney *et al.* (177) found that acetylcholine accelerated denervated atropinized dog hearts and attributed the result to a direct stimulating effect on the heart tissue, or on intracardiac-cardioaccelerator ganglion cells (178). A third laboratory (179) also reported that acetylcholine, after a transitory slowing and weakening of the heart, caused a prolonged increase in activity. Subsequently they found that minced rabbit or cat heart treated with acetylcholine produced a substance (believed to be epinephrine or an altered acetylcholine) which inhibited atropinized rabbit intestine (180).

Acetylcholine was reported to be inconstant in the control of cardiac irregularities induced by epinephrine (181). However, in-

travenous acetylcholine was found to stop all eighty-one attacks of paroxysmal ventricular tachycardia in a patient (124).

Cocaine prolongs the duration of cardiac responses to epinephrine in the adrenalectomized vagotomized and sympathectomized anaesthetized dog and cat, apparently by inhibiting its destruction (182).

#### METABOLISM

Acetic acid is readily converted to carbon dioxide by the isolated mammalian heart (183), but the heart is unable to split glycine to carbon dioxide (184). In the absence of substrate the oxygen uptake of heart muscle slices from rats in the terminal stage of hemorrhagic shock was reduced. However, when pyruvate is added, the oxygen uptake is increased over normal. There is therefore no evidence that in shock the heart has an impaired ability to oxidize pyruvate (185). The dry weight of the normal heart was found to be about one-fifth of the wet weight and this is unchanged in shock (186).

In experimental ketosis, a direct correlation was found between the blood ketone level and cardiac glycogen, but no such relation was seen for blood sugar and blood lactic acid (187). In the B<sub>1</sub> avitaminotic rat, electrical systole is prolonged and cardiac glycogen is reduced (188). A report on the action of large doses of insulin on the rabbit heart has appeared (189). After intravenous injection, cytochrome C is taken up by the heart, liver, and kidneys, increasing the arteriovenous difference in anoxia through an augmentation in tissue uptake of oxygen (190).

*Deficiencies.*—Potassium deficient diets in the rat resulted in widespread edema and pathological changes in the heart as well as in other organs. Atrial and ventricular myocardial necrosis was followed by leucocytic infiltration and finally by lightly collagenized scars. Large atrial mural thrombi were seen occasionally with early lesions (191).

Chronic thiamine deficiency in rats caused bradycardia, reducing the heart rate from a normal of about 450 to about 275. Sinus arrhythmia, atrial fibrillation, auriculo-ventricular nodal rhythm, sinus arrest, shifting pacemaker, first degree auriculo-ventricular block, auricular, nodal, and ventricular ectopic beats and prolonged QRS complexes were also seen. These defects were partially or completely corrected after thiamine therapy. In many

rats there were pathological changes in the atrial myocardium and pulmonary veins (192). In pigs with avitaminosis there was a prolonged auriculo-ventricular conduction time. QRS was occasionally dropped when these animals became excited, after the exhibition of atropine and when acute cardiac failure developed (193).

Twelve out of a total of twelve thousand medical admissions in a Cincinnati hospital were diagnosed as having beriberi heart disease (194), and criteria for the diagnosis of beriberi heart disease were proposed (195). A pellagrin with low voltage and flattened  $T_1$ ,  $T_2$  and  $T_3$  responded to therapy with N-methylnicotinamide with an increase in voltage and augmentation of the T waves. Two months later with continued therapy the patient had a normal electrocardiogram (196).

*Thyroid.*—The sudden death (attributed to heart failure) which was seen in thyroid-fed rats was prevented by the feeding of yeast. However, thiamine, riboflavin, pyroxidine, pantothenate, choline, and 2-methylnaphthaquinone did not prevent this (197). In rats on a high thyroid diet, left ventricular predominance, ventricular premature systoles, and occasional dropped beats were seen, and at necropsy small scars with loss of muscle fibers and histological cellular infiltration were observed (197).

Raab felt that since thyroid sensitizes the heart to epinephrine and since thyroidectomy or thiourea reduce this sensitivity it would follow that a decrease in thyroid activity would be useful in the treatment of angina pectoris (198). However, the results obtained in ten cases were not striking, and the invoking of an epinephrine link in the chain of events is not convincing. Thyroidectomy caused a slowing of the heart rate and decreased voltages in the electrocardiogram. Treatment with thiourea caused somewhat similar effects (199). Excessive self administration of thyroid extract in a case of attempted suicide caused T waves of definitely decreased amplitude, and this was reversed when thyroid was withdrawn. Although the heart rate in this thyroid fed subject was only 85 at rest, excitement and effort readily produced tachycardia which subsided only with prolonged rest (200). In confirmation of Evans (201) thyroidectomy was found to lead to an increase in cardiac glycogen (202). Thyroidectomy seems to affect the metabolism of the heart muscle independent of its depression of general metabolism (202).

## DIGITALIS, QUINIDINE, AND RELATED DRUGS

A monograph on digitalis and other cardiotonic drugs has appeared (203). Several reports on the clinical use of the glycosides and their toxic action in myocardial infarction (204), in auricular fibrillation (205, 206, 207) and in producing sinoauricular block (208) have been published.

Intravenous injection of atabrine into the dog heart-lung preparation leads to heart failure with a fall in cardiac output and rise in venous pressure. The addition of strophosid was found to cause almost complete recovery of these functions in doses which do not change the heart rate and do not lead to irregularities of the heart rhythm. The cardiac glycosides are reported to effect a prompt and long-lasting improvement of the work capacity of the failing heart. This is in accord with the finding that fatigued papillary muscles responded more readily to cardiac glycosides than did nonfatigued muscle (209). This improvement is a function of the concentration of glycoside. The dog heart binds only an amount of glycoside equal to the minimal lethal dose regardless of the concentration in the blood. The rate of uptake is proportional to the blood concentration (210, 211). The cardiac slowing seen after digitalis administration is not due to the inhibition of acetylcholine esterase (212).

The amount of digitalis required to cause death in experimental digitalis poisoning is reported to be equal to the dose required to produce auriculo-ventricular dissociation plus a constant quantity (213). It is reported that digitalis in toxic doses produced myocardial lesions in the dog. Atropine, aminophyllin and theobromine appeared to modify this effect, while papaverine did not (214). This is in serious contradiction to earlier experimental and clinical experiences, and deserves attempts at confirmation. Cerberin, a glycoside from the East Indies, appears useful in the control of auriculo-fibrillation (215). The use of quinidine and its derivatives in the treatment of ventricular paroxysmal tachycardia is the subject of several reports (216 to 219).

Among the substances reported to increase the contractile force of the heart are coumagine (65) and the erythrophleum alkaloids (221).

## CORONARY BLOOD FLOW

The subject has been extensively reviewed by Gregg (222).

The functional sequences of coronary occlusion have been discussed by Wiggers (223). The subject of coronary occlusion continues to receive considerable attention in clinical reports (224 to 229).

Anginal pain occurred in the right arm in a case with dextrocardia and situs inversus (230). It also appeared during stimulation of the hyperactive carotid sinus in three subjects (231, 232). It occurred after direct trauma to the chest (233) and after a bullet passing into the mediastinum (234). Confirmation is forthcoming of the beneficial effects of paravertebral block (235) and of intravenous papaverine (236) in the management of angina pectoris. An experience in favor of stress as a cause of myocardial infarction is reported. Eleven soldiers developed acute myocardial infarction subsequent to strenuous effort, a result attributed to ischemia, to subintimal hemorrhages, or to rupture of atheromatous abscesses (237). Subjects who breathed 10 per cent oxygen and 90 per cent nitrogen mixtures showed electrocardiographic changes indicating coronary insufficiency (238).

The frequency of combined vascular lesions of brain and heart is far greater than would be expected on a basis of chance (239). This suggests that a generalized tendency to thrombo-embolic phenomena may be the etiological factor in these vascular accidents.

In a series of dogs with ligated left anterior descending coronary arteries, those receiving papaverine appeared to have smaller infarcts than untreated animals or those receiving aminophyllin (240). Neither atropine, testosterone, nor pitressin appeared to affect the size of the infarct (240). Increased coronary blood flow of the dog heart-lung preparation was reported with the use of dimethylaminoethanol (241) and of adenosine triphosphate (57).

The flow of blood into the left anterior atrial artery was measured phasically and qualitatively in the dog heart-lung preparation. Forward flow into the atrial artery occurred during both ventricular systole and diastole with abrupt, momentary interruption of inflow at the onset of ventricular systole and diastole. Elevation of tension within the left atrium diminished atrial arterial inflow. It is suggested that interference with the atrial blood supply may enhance ectopic atrial mechanisms (242).

Bilateral cervical vagotomy did not abolish the electrocardiographic alteration produced by pulmonary emboli, nor could evi-



dence be obtained to support the contention that a pulmonocoronary reflex via the vagus produced coronary insufficiency in experimental pulmonary embolism. It is shown that dynamic alterations alone are adequate to account for the coronary insufficiency encountered in pulmonary embolism (243). This supports earlier studies of this group (244) and suggests that the widespread teaching that the vagi are coronary artery constrictors is erroneous.

#### METHODS

A double lumen flexible radiopaque catheter has been devised for simultaneous sampling of blood or of pressure pulses of the right heart (245). Some distortions of the pressure pulses are to be expected with this method because of the distensibility of the catheter and on account of movements of the catheter during the heart cycle. Utilization of the Hamilton manometer for simultaneous recording of the pressures in the thoracic cavity, right heart, and systemic arteries has been reported (246).

A simple method has been developed for the orthodiographic measurement of the transverse diameter of the heart by means of the simple fluoroscope (247). Electrocardiograms of heart border motion have been recorded by the use of the fluoroscope and a 931-A multiplier phototube. Various points on the border of the heart were shown to have their own characteristic motions and these changed definitively in the presence of cardiovascular disease (248). This method appears to be superior to roentgenkymography.

An instantly recording cardiometer which counts the R waves of the electrocardiogram is described (249). An objective simple direct procedure to test the circulation time from lung to ear has been devised (250). The subject takes a single rapid deep inspiration of pure nitrogen and the time required for a change in the color of the ear as measured by the oximeter is determined.

The technique for a heart-lung preparation in the rat is described (69). A method of visualizing the coronary arteries in a living animal is presented (251). The strain gauge (252) has been utilized for the measurement of blood pressure. The electromagnetic flowmeter has been modified to utilize an alternating induction field which is reported to give high sensitivity for the flow measurement (253).

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CARDIOVASCULAR DEPARTMENT  
RESEARCH INSTITUTE  
MICHAEL REESE HOSPITAL  
CHICAGO, ILLINOIS

## DERIVATIVES OF BLOOD PLASMA

BY DWIGHT J. MULFORD

*Department of Physical Chemistry, Harvard Medical School and Massachusetts  
Department of Public Health, Boston, Massachusetts*

*Introduction.*—Ever since the beginning of the last century it has been known that blood plasma is a complex system containing a large number of protein components. Since as far back as 1852, neutral salts have been employed in fractional precipitation of proteins involved in such a system (1,2). For the fractionation of proteins by salting out procedures it was necessary that characteristics of the neutral salts, as well as the protein, be known (3 to 10). Chick & Martin (11) in 1913 and Sørensen (12) in 1925 stated that ammonium sulfate, if added in sufficient amount, would cause the precipitation of practically all the plasma proteins. Cohn (13) reported that the solubility of a purified protein in a concentrated salt solution was not only dependent upon the characteristic of the salt used, but also upon the ionic strength, pH, and temperature of the mixture.

Other procedures for protein separations have been developed as the knowledge of protein behavior has progressed. Dialysis and electrodialysis at reactions close to the isoelectric points of the various plasma proteins have been used to precipitate those proteins insoluble in water. Ferry, Cohn, & Newman (14) were able to maintain egg albumin, a readily denatured protein, in ethanol-water mixtures at  $-5^{\circ}\text{C.}$  for long periods of time, then removed the ethanol before the temperature was raised, and recrystallized the unmodified protein. Further, the Harvard group studied the effect of neutral salts on the solubility of proteins in ethanol at low temperatures (15, 16, 17). They found that the solubility was increased with increasing amounts of salt in much the same way as is the solubility of globulins in water.

*Factors affecting protein solubility.*—The separation of the many proteins of a biological fluid has been accomplished by control of their relative solubilities in a system of many variables. The number of variables required depends upon how many proteins are to be separated and how nearly alike are their physical chemical properties. Usually, conditions are set up such that the protein to be separated has (a) a high solubility when most other components of

the system have low solubilities, or (b) a low solubility when most of the components of the system have high solubilities.

Certain proteins can be separated in aqueous systems because of their insolubility at their isoelectric points. Of these, those termed globulins are readily dissolved by neutral salts. The influence of ionic strength is so great that fractions of serum globulin with a given solubility in water are about one thousand times more soluble in a dilute solution of sodium chloride. The quantitative separation of proteins of this kind has thus depended upon the removal of salt by dilution, dialysis, and adjustment of the pH to the isoelectric zone.

Proteins can be separated easily if they have different isoelectric points, different solubilities in the absence of salt, and different activity coefficients in the presence of salts, especially at very low salt concentrations. The point of maximum precipitation of proteins tends to become sharper in a system with decreasing salt concentrations. Various outside influences may affect the point of maximum precipitation in such a way that the protein is more insoluble at a pH quite removed from the isoelectric zone. The presence of another protein with a slightly different isoelectric point tends to shift the pH of maximum precipitation toward the isoelectric point of the second protein, and the precipitate resulting is a complex of both proteins.

In order to achieve satisfactory separation between such proteins, it is necessary to approach the precipitation zone of all the proteins from the same side of their respective isoelectric points. In other words, if a mixture of proteins A, B, and C have isoelectric points of 6.2, 5.8 and 4.0 respectively, one approaches the precipitation zone from a pH either above 6.2 or a pH below 4.0.

In all but the very few proteins that have been separated and purified from all other proteins, solubility is influenced by the nature and the amount of protein in the system. Four variables thus determine solubility in such systems: (a) pH; (b) salt concentration; (c) temperature; and (d) the protein concentration. If the temperature and the amount and nature of the protein in the system are maintained constant in the range of low ionic strengths, an increase in salt concentration at any pH increases the solubility of the protein. Globulins are known, however, with solubilities which are increased on one side of the isoelectric point by salt, but are diminished on the other side (6, 18, 19, 20, 21). The system of

fractionation reported in this review involves lowering the solubility of proteins with ethanol in the range of low temperatures and low ionic strengths. The introduction of ethanol for the precipitation of proteins increases the number of variables from four to five. The methods of fractionation of plasma in ethanol-water mixtures were based on previous studies upon the solubility of amino acids, peptides and proteins in similar systems (22 to 31). Cohn *et al.* (16) described a method for the fractionation of beef plasma into fibrinogen,  $\alpha$ -,  $\beta$ -, and  $\gamma$ -globulins, and albumin. Ethanol concentrations ranging from 10 per cent to 40 per cent were used with low temperatures ranging from 0°C. to -5°C. Many of the protein fractions prepared by this method were investigated for use in transfusions at the Peter Bent Brigham Hospital, Boston, and for their antibody content at the Massachusetts State Antitoxin and Vaccine Laboratory. It is the purpose of this review to discuss more recent developments in ethanol fractionation with respect to those physiologically active components which are associated with the proteins that have been separated in relatively pure form.

*Large scale protein separation using ethanol at low temperatures.*—During the war, normal human plasma was fractionated on a large scale. Approximately 140 to 240 blood donations collected by the Boston Red Cross each week for three years were used in the Harvard Pilot Plant for the development of the ethanol procedure. Soon, seven industrial houses of the country were given Navy contracts for the production of the clinically usable proteins obtained from plasma by ethanol fractionation. By VJ Day plasma from over 2,000,000 donors had been fractionated.

In general, the procedure as discussed by Cohn *et al.* (32) was one in which fibrinogen,  $\beta$ -, and  $\gamma$ -globulins were precipitated into Fraction I;  $\alpha$ -,  $\beta$ -, and  $\gamma$ -globulins into Fraction II + III; the remainder of  $\alpha$ - and  $\beta$ -globulins into Fraction IV, and albumin into Fraction V. Although the amount of protein contributed to each precipitate by occluded supernatant solution was too small to necessitate repeated washings of the fractions, the occluded ethanol had to be removed before the proteins could be exposed to temperatures above that employed for fractionation. This was accomplished conveniently and completely by drying from the frozen state under reduced pressure (33 to 39). The paper by Cohn *et al.* (40) described and discussed each method developed for the separation of the various plasma proteins into fractions. Table I shows

the fractions separated from plasma by Method 6 and the amount of the various electrophoretic components contained in each per liter of plasma. The supernatant fluid remaining after the removal of Fraction IV was further freed of globulins by filtration, as globulin in Fraction V lowered the heat stability of the final albumin. It was observed, however, that these conditions for precipitating Fraction IV were such that some of the proteins in IV were being denatured. This was largely taken into account in the development of Method 6, and it was found that conditions not causing denaturation, namely the use of 18 per cent ethanol and pH 5.2, could be used instead of 40 per cent ethanol and pH 5.8. The remainder of the globulins in the supernatant fluid were still removed when the ethanol concentration was 40 per cent and the pH 5.8 as in earlier procedures, although efforts to reduce the maximum ethanol concentration to 25 per cent continue.

TABLE I  
DISTRIBUTION OF PLASMA PROTEINS BY METHOD 6 (40)

<i>Fraction</i>	<i>Albumin</i>	$\alpha$ -	$\beta$ -	$\gamma$ -	<i>Fibrinogen</i>	<i>Total</i>
grams per liter of plasma						
I	0.2	0.3	0.5	0.3	2.1	3.4
II+III	0.8	1.1	9.1	7.0	1.0	19.0
IV-1	0	4.5	0.5	0.1	0	5.1
IV-4	0.9	2.7	2.2	0	0	5.8
V	29.9	1.3	0.3	0	0	31.5
VI*	0.8	0.2	<0.1	0	0	1.0
Total	32.6	10.1	12.6	7.4	3.1	65.8

\* Mother liquor following precipitation of Fraction V.

The procedure, Method 6, and the various procedures for sub-fractionation will be described in the following sections discussing the physiological importance of each fraction.

### FRACTION I

*Chemical procedure.*—Normal human plasma containing 0.8 per cent sodium citrate was stirred gently in a cold room until the temperature of 0°C. was reached. To each liter was added a mixture of 53.3 per cent ethanol and acetate buffer pH 4, to bring the ethanol concentration to 8 per cent (volume per cent as measured at +25°C.) and pH to  $7.2 \pm 0.2$ . During the addition the tempera-

ture was lowered gradually to  $-2.5^{\circ}\text{C}$ . The ionic strength<sup>1</sup> for precipitation was 0.14 and the protein concentration 5.1 per cent.

The precipitate formed was Fraction I and electrophoretically contained 15 per cent  $\beta$ -globulin, 9 per cent  $\gamma$ -globulin, 9 per cent  $\alpha$ -globulin, 6 per cent albumin, and about 62 per cent fibrinogen (Table I). It has been prepared as a dry sterile powder by resuspending the wet precipitate in citrate solution, filtering under sterile conditions and drying from the frozen state under reduced pressure (41). In this condition the fibrinogen was quite stable for long periods of time, the dry powder readily dissolving on the addition of water. The resulting solution could be promptly clotted on the addition of thrombin.

*Preparation and properties of fibrin films.*—Fraction I has been the raw material for study by several investigators. Ferry & Morrison (42, 43, 44) used it in their studies on the production of fibrin plastics; they produced clots having a wide variety of physical characteristics by mixing thrombin and this fraction. Two types of clots were prepared, namely type A (fine) and type B (coarse). Type A clots, which were produced at alkaline reaction, were transparent, gelatinous, and friable. Their tensile strength and maximum elongation were low. They did not synerize readily, but did adhere well to surfaces upon which they were formed. Type B clots, however, which were produced at acid reaction were opaque, doughy, and nonfriable. They had a high elongation and plastic flow, but did not adhere well to surfaces upon which they were formed. They synerized very readily. Two papers by these workers (43, 44) on the conversion of fibrinogen to fibrin clots and the methods for preparation of fibrin films are soon to be published.

*Clinical value of fibrin films.*—Type B (coarse) clots which have been converted to films by Ferry & Morrison (42, 43, 44) have proved of much value to the surgeon. A series of papers by Ingraham and Bailey (45 to 48) report that films were satisfactory for the repair of dural defects and the prevention of adhesions between damaged nerve tissue and adjacent structures. This was true not only in monkeys, but also in humans.

<sup>1</sup> Throughout this discussion, the ionic strength ( $\Gamma/2$ ) is calculated from the relation  $\Gamma/2 = 1/2 \sum c_i z_i^2$ , where  $c_i$  is the concentration of the  $i$ 'th ion in moles/l.,  $z_i$  is its valence, and the sum is extended over all the ions in the system. For a univalent electrolyte, such as sodium chloride, the ionic strength is equal to the concentration in moles/l.

Morrison & Singer (49) studied the rate of absorption of fibrin films in the rabbit. Various types of films were implanted subcutaneously and intramuscularly in the rabbit and held in place by silk sutures. They found that absorption was complete in from nine to eighty-one days depending upon the kind of film. Heat sterilized films are much more resistant to enzyme action and are absorbed much more slowly. All films studied were of the same dimensions but varied in weight from 4 to 11 mg. fibrin per square cm. The studies of Bailey & Ingraham (45) indicated that the absorption of films in humans was qualitatively similar to that in rabbits.

Other possible uses of fibrin films have been studied. Fibrin films and fibrin tubes have been suggested by Singer (50) for the end-to-end sutures of nerves on the basis of experiments in animals. Swenson (51) has attempted the use of fibrin tubes in the anastomosis of blood vessels with some success.

*Preparation and properties of fibrin foam.*—A second fibrin product produced by Bering (52) has been demonstrated conclusively to have much clinical value. Bering called the material fibrin foam and prepared it by beating fibrinogen into a foam either by using a Waring blender or an egg beater. The foamy fibrinogen was clotted by the addition of thrombin and the clot resulting was dried from the frozen state under reduced pressure. The resulting dried product had the appearance of a dry sponge and absorbed solutions very well. It was sterilized by dry heat at 170°C. for a period of several hours. This heat treatment also modified the physical properties of the fibrin and made it more adhesive to the tissues to which it was applied.

*Clinical value of fibrin foams.*—Bailey & Ingraham (53, 54) have reported on the importance of fibrin foam for the control of oozing of blood from the dura of both monkeys and humans. Sterile fibrin foam previously soaked in a solution of sterile thrombin was placed on the bleeding dural area. Clotting of the patient's fibrinogen resulted immediately, and the oozing stopped. Of particular importance was the use of foam in brain surgery where there was oozing from the outer surface of the dura, the beds of central nervous system tumors, and brain substance, as well as venous bleeding from spinal veins, superficial cerebral veins and dural sinuses. Bailey & Ingraham reported that tissue reactions were



minimal in all animals examined histologically. They used foam in 170 neurosurgical patients under varying conditions and stated that its hemostatic action had shortened their operations markedly in most cases.

Fibrin foam and thrombin have been studied in general surgery and have been proven of value by Bailey *et al.* (55). Others have reported success in controlling hemorrhage from the cut surface of the liver and kidney, and in prostatectomies (56). Losch (57), furthermore, has demonstrated its valuable effect in certain dental operations, especially in hemophilia.

*Factor lowering clotting time of blood in hemophilia.*—A factor concerned in the blood clotting of hemophiliacs has been found in Fraction I. It may be identical with the substance which was observed in normal human plasma by Patek & Taylor (58) and in beef and swine blood by Adams & Taylor in 1937 (59). The procedure for human plasma fractionation did not concentrate all the antihemophilic material into Fraction I, however, as Taylor *et al.* (60, 61) have demonstrated concentration of activity not only in Fraction I but also in Fraction III-2, another fraction obtained from plasma.

The antihemophilic activity which Howell (62) called plasma thromboplastin is associated with the euglobulins of plasma (58, 63). A separation of the activity from fibrinogen is being attempted, though thus far without success. Experiments in progress suggest that this active component is a separate protein. Studies are being made both at Harvard and the Massachusetts Blood Processing Laboratory to answer this question.

Lozner, Kark, & Taylor (64) demonstrated in 1939 that normal human serum caused a marked decrease in clotting time when given either by intravenous or intramuscular injection into patients with hemophilia. Doses of 200 mg. of Fraction I resulted in decreases in clotting times from around fifty to one hundred minutes to around ten minutes. In one instance, a dose of 11.5 mg. of protein gave a drop from ninety-five minutes to forty-five minutes. Larger doses resulted in a fall to levels approximating normal clotting times. The duration of the response was generally six to eight hours with the 200 mg. doses and only slightly longer for large doses. Thereafter the clotting time gradually lengthened, until after twenty-four hours it had returned to the preinjection

value. In comparison to whole blood and plasma 200 mg. of Fraction I were equivalent to 100 cc. of fresh whole blood and 80 cc. of frozen plasma.

It has been demonstrated that the antihemophilic activity is highly labile, disappearing rapidly at room temperature in both plasma and Fraction I solutions. Stored at  $-5^{\circ}\text{C}.$  to  $+5^{\circ}\text{C}.$ , Fraction I in solution or as a wet paste showed a decided loss in antihemophilic activity, but stored at low temperatures and in the dry state, it lost very little of its activity.

### FRACTION II+III

*Chemical procedure.*—Following the removal of Fraction I, the supernatant fluid that remained was adjusted to 25 per cent ethanol, pH 6.8, ionic strength 0.09, and protein concentration 3 per cent. The temperature was lowered to  $-5^{\circ}\text{C}.$  and a second precipitate, Fraction II+III, was removed. In the earliest methods Fraction II and Fraction III were removed as separate fractions from plasma. Fraction II was removed first, usually at pH 7.4, ionic strength 0.04,  $-5^{\circ}\text{C}.$ , and at 15 per cent ethanol. Fraction III was then removed by a change of ionic strength to 0.02 and of ethanol concentration to 25 per cent. It was found that several components especially prothrombin and various antibodies, were present in both fractions. As a result, the latest procedures removed Fraction II and Fraction III in one precipitation, the fraction being known as Fraction II+III. As determined by electrophoresis, Fraction II+III was 4 per cent albumin, 6 per cent  $\alpha$ -globulin, 48 per cent  $\beta$ -globulin, 37 per cent  $\gamma$ -globulin, and 5 per cent fibrinogen (Table I). This fraction contained most of the plasma antibodies, prothrombin, the isohemagglutinins, and a precursor of the fibrinolytic enzyme. In addition, some of the proteins occurred as conjugated proteins, the prosthetic groups being cholesterol, carotenoid pigments, phospholipids, and carbohydrates.

For the purification of these active components, Oncley *et al.* (65) studied various methods of subfractionation of Fraction II+III. By their methods they were able to separate each of the above active components into further subfractions. In the subfractionation of the physiological components of Fraction II+III several precipitates which contained a high percentage of  $\beta$ -globulin were obtained. Among them were Fraction III-0, III-1, III-2 and III-3.  $\gamma$ -globulin was largely but not entirely concentrated in Fraction

II, though a large amount of the  $\gamma$ -euglobulins especially of animal plasmas, separate in III-1. Since the  $\alpha$ - and  $\beta$ -globulins were precipitated before the  $\gamma$ -globulins, the methods of subfractionation of II+III appeared to remove precipitates in the reverse order. Those designated as III were removed first and those designated as II were removed last.

Method 9 of Oncley *et al.* (65), appeared to yield fractions of proteins which are in their native state. Earlier procedures gave some fractions that were native, but others that were denatured. The following discussion concerns for the most part the various fractions as they were obtained by Method 9.

*Chemical procedure for subfractionation.*—The first step in the subfractionation of Fraction II + III was the removal of lipoprotein impurities by washing the fraction with 20 per cent ethanol at pH 7.2, ionic strength 0.005, temperature  $-5^{\circ}\text{C}$ , and 1.0 per cent protein concentration. The portion which remained insoluble under these conditions and which was known as Fraction II+III W consisted of practically all of the  $\gamma$ -globulin, blood grouping globulins or isohemagglutinins, and the prothrombin originally present in Fraction II+III. The soluble portion contained most of the lipid and carotenoid pigment originally in Fraction II+III and these were precipitated by adjusting the solution to 25 per cent ethanol and pH 5.7. The precipitate removed was designated as Fraction III-O.

*Characteristics of lipoproteins of Fraction II + III.*—A considerable amount has been learned already concerning the chemistry and physiology of Fraction III-0. Chemically, it contained a lipoprotein which in the ultracentrifuge behaved like the "X protein" of plasma first described by McFarlane (66) and later by Pedersen (67). By electrophoretic analysis, it contained largely  $\beta$ -globulin. Fraction III-0 has been demonstrated further to contain inhibitors of both the fibrinolytic enzyme which occurs in plasma, and of thrombin (68). An inhibitor of thrombin has been found also in the other lipid rich fraction IV-1, another of the main plasma fractions (Table I). Unpublished reports have indicated that vitamin A (69) was concentrated in Fraction III-0. Recent work has demonstrated the presence of an estrogen in this fraction (70, 71).

*Separation of isohemagglutinins, prothrombin, fibrinolytic enzyme from  $\gamma$ -globulin.*—For the separation of the isohemagglutinins, prothrombin and the fibrinolytic enzyme from  $\gamma$ -globulin, Fraction

II+III W was resuspended in an acetate buffer and adjusted to pH 5.2, ionic strength 0.015, 17 per cent ethanol, 1.2 per cent protein, and  $-6^{\circ}\text{C}$ . The precipitate removed by centrifugation was Fraction III-1,2,3. Resuspending this in water and adjusting the conditions to pH 5.4, ionic strength 0.08, ethanol 1 per cent, protein concentration 2.4 per cent, and  $+1^{\circ}\text{C}$ . resulted in the precipitation of Fraction III-2,3 which contained prothrombin and the precursor of the fibrinolytic enzyme.

*Separation of fibrinolysin and prothrombin.*—Fraction III-2,3 contained that amount of fibrinogen which was not removed in Fraction I, the first major fraction removed from plasma. On the addition of thrombin to the suspension of Fraction III-2,3 which had been adjusted previously to pH 6.8 or 7.0 with sodium glycinate buffer, a clot formed which was removed by centrifugation. The clot was designated as III-3 and contained a high level of fibrinolytic enzyme precursor. The supernatant fluid remaining after the clot was removed was Fraction III-2. It proved to be the richest source of prothrombin and could be dried as such or converted to thrombin by the addition of thromboplastin and calcium chloride and then dried.

*Activity and use of thrombin.*—The fractionation for prothrombin by Richert (72) proved to be difficult as the stability was poor even in the dry state. Seegers, Loomis & Vanderbelt (73) have recently obtained bovine prothrombin in what appears to be pure form. Thrombin, on the other hand, produced from Fraction II+III by Method 9 of Oncley *et al.* (65), although not as active as that by Seegers (74, 75, 76), is of human material and has proven most satisfactory as a hemostatic agent and in the making of fibrin films and foams described earlier. Further, it has been used along with fibrinogen in skin grafting by Cronkite, Lozner & Deaver (77) and in burns by Hawn *et al.* (78). An excellent review on blood coagulation has been published just recently by Ferguson (79).

*Components of complement.*—Pillemer & Mulford (80) found that one of the four components of complement, C'1, occurred almost completely in Fraction II+III, and the bulk of the evidence indicated that this substance was concentrated in Fraction III-2. A second component, C'2, was found in the supernatant after the removal of Fraction II+III and was precipitated in Fraction IV-1, a later fraction. The other two components, C'3 and C'4, have

thus far not been accounted for and may have been destroyed during fractionation.

*Purification of fibrinolytic enzyme (plasmin).*—Fraction III-3 was found to be an excellent source of the fibrinolytic enzyme called plasmin by Christensen & MacLeod (81) and serum tryptase by Ferguson (79). In the plasma it occurred in the inactive form, plasminogen, which could be activated rapidly by streptokinase (streptococcal fibrinolysin). Richert (82) found that a large portion of the inactive form could be activated spontaneously on standing under sterile conditions. He was able to concentrate plasmin by adsorbing it on fibrin formed by adding thrombin to a suspension of Fraction III-2,3, which usually contained fibrinogen. The fibrin clot was liquified by the enzyme itself on standing. The resulting solution was sterilized and preserved in highly active form. Nothing is thus far known about its clinical uses.

*Precipitation of the isohemagglutinins from group specific bloods.*—In Method 9, the supernatant fluid resulting from the precipitation of Fraction III-2,3 contained the isohemagglutinins or the blood grouping globulins. They were separated into Fraction III-1 by the adjustment to pH 6.3, 15 per cent ethanol, and ionic strength 0.06, protein concentration 0.5 per cent, and temperature  $-5^{\circ}\text{C}$ . Pillemer (83) was the first to accomplish a separation when he used small scale experiments to remove the anti-A agglutinin from B bloods. His collaboration with the Harvard group led to Method 6 in which Fraction II+III was suspended in a large volume of water to an ionic strength of 0.005. The pH was adjusted to 6.3, and the isohemagglutinin fraction precipitated at room temperature. In this procedure the  $\gamma$ -globulin and thrombin were sacrificed. Each procedure for the preparation of the isohemagglutinins, whether it was Method 6 or Method 9, was of no value on Fraction II+III from plasma pools of mixed blood types, as no procedure has been developed to separate anti-A from anti-B isohemagglutinins. If, however, the pools of plasma came from either Group A bloods or Group B bloods, the anti-B and anti-A blood grouping material, respectively, could be prepared by either Method 6 or Method 9. Pillemer *et al.* (84) reported on the separation and concentration of the isohemagglutinins from group specific human plasma. Using Method 6 they were able to concentrate the active material to sixteen times more than plasma activ-

ity. Their investigations required the typing of donors at the donor clinic, and all A blood donations were kept for an A plasma pool and all the B blood donations for a B plasma pool. Certain standards were required to insure against the possibility that a recipient be transfused with an incompatible blood. Titers of the various preparations were checked by several investigators and the results were recorded by DeGowin (85). Recent studies have demonstrated that the Rh typing material can be prepared by a similar procedure (86).

In a normal distribution of population the blood types occur as follows: 45 per cent are Group O; 41 per cent are Group A; 10 per cent are Group B; and 4 per cent are Group AB. On this basis the amount of anti-A material that was obtained from Group B bloods was quite small in comparison to the anti-B material obtained from Group A bloods.

*Preparation of isohemagglutinins from Group O bloods.*—Melin (87) investigated the possibility of using Group O bloods which contained both anti-A and anti-B isohemagglutinins. He reported that the anti-A substance could be prepared from a Fraction II+III which had been separated from a pool of plasma obtained from a mixture of both Group O bloods and Group B bloods.

The principle involved was one of absorption in which the anti-B material in O bloods was absorbed on the B cells. Several precautions in the absorption were necessary. These included the accurate typing of each bottle of blood, as even one Group A blood in a large pool of Group O and Group B decreased markedly the yield of anti-A in the final product; the length of time the O bloods were exposed to the B cells; and the temperature at which the absorption was carried out. Melin (87) found that the ratio of B bloods to O bloods that occurred normally gave the maximum absorption of anti-B such that the remaining plasma was extremely low in anti-B. A second absorption of the final material was suggested to insure that there was no impurity of anti-B in the anti-A product distributed for typing. By a like procedure anti-B material may presumably be obtained using Group O bloods with Group A bloods, if for any reason this were desirable. Against A<sub>1</sub> cells the anti-A material prepared by mixing O bloods with B bloods was as good as the anti-A prepared from Group B bloods alone. Furthermore, against A<sub>2</sub> cells it was superior to that prepared from Group B bloods. Kramer (88) sug-

gested this when he found that O serum usually agglutinated A<sub>2</sub> cells more readily than did B serum and that O serum had nearly the same titer against A<sub>1</sub> cells on the one hand and A<sub>2</sub> cells on the other. Furthermore, the procedure by Melin is more economical than that using just Group A or Group B bloods.

*Precipitation of  $\gamma$ -globulins.*—The chemical studies of various of the fractions of plasma were carried out in several laboratories. Williams *et al.* (89) reported on electrophoretic and ultracentrifugal studies of solutions of human serum albumin and immune serum globulin. On 162 preparations delivered to the armed forces by the commercial houses, albumin was routinely concentrated by ethanol fractionation from 55 per cent in plasma to 98.5 per cent. Further, the immune serum globulin of thirty-eight preparations showed that the  $\gamma$ -globulin was increased from 11 per cent in plasma to about 85 to 95 per cent in the final product.

In general the yield of  $\gamma$ -globulin in Fraction II was only about 50 per cent of that in plasma, the remainder being previously precipitated in Fraction III-1. In Method 9 for the subfractionation of Fraction II + III as developed by Oncley *et al.* (65) part of the  $\gamma$ -globulin that came down in Fraction III-1 in earlier methods was obtained in Fraction II-3. Recent studies by Deutsch, Petermann & Williams (90) have demonstrated that the yields of  $\gamma$ -globulin can be increased by a pepsin digestion of fractions containing both  $\beta$ - and  $\gamma$ -globulins. Fraction III-1 was partially digested with pepsin at pH 3.5 and this was followed by fractional precipitation with ethanol. The above workers were able to take advantage of a low ionic strength of 0.029 for the separation, a fact they recognized as being very important in the separation of  $\beta$ - and  $\gamma$ -globulins. Further studies of Petermann (91) and Bridgman (92) have demonstrated the effect of various proteolytic enzymes on  $\gamma$ -globulin, and it was indicated that under controlled conditions papain or bromelain (91) could split the globulin into particles of one-quarter size and that pepsin (92) could split it into particles of one-half size. Immunological assays indicate that, although 60 to 90 per cent of the globulin molecules had been split, only a small amount of the antibody activity was lost with the exception of typhoid "O" agglutinin which was completely destroyed by pepsin.

Further studies by Deutsch *et al.* (93) concerning the effect of ionic strength on the separation of  $\beta$ - and  $\gamma$ -globulins indicated that the solubility of  $\gamma$ -globulin was influenced markedly by the



ionic strength. A separation was possible if the ionic strength was as low as 0.01 for under the conditions studied  $\gamma$ -globulin was soluble at that concentration. If, however, the ionic strength was 0.04  $\gamma$ -globulin was insoluble and a separation could not be made. By applying a pH of 5.1, ionic strength 0.01, ethanol concentration 17 per cent, and temperature  $-6^{\circ}\text{C}$ . to Fraction II+III they were able to separate the  $\gamma$ - from  $\beta$ -globulin with a recovery of 75 per cent of the total  $\gamma$ -globulin in a single step without sacrifice in purity. In a similar way the development of Method 9 by Oncley *et al.* (65) has increased the yield of  $\gamma$ -globulin.

The protein in the supernatant fluid after the precipitation of Fraction III-1,2,3 was largely  $\gamma$ -globulin, a part of which was removed by adjusting the supernatant III-1,2,3, to pH 5.2 and 17 per cent ethanol, ionic strength 0.05 and temperature  $-6^{\circ}\text{C}$ . The precipitate recovered by centrifugation was known as Fraction II-3. The fraction was predominantly the least soluble  $\gamma$ -globulin which in earlier methods of subfractionation was contained in Fraction III-1 and not previously used in clinical studies. The immunological titers of the antibodies in Fraction II-3 were almost identical with those of the more soluble Fraction II-1,2 the next fraction removed in Method 9 and the fraction having most study with respect to its immunological activity and clinical use.

To precipitate the most soluble Fraction II-1,2 which contained the remainder of the  $\gamma$ -globulin, the supernatant of Fraction II-3 was adjusted to pH 7.4 and 20 to 25 per cent ethanol using sodium bicarbonate and 95 per cent ethanol which had been previously cooled. Fraction II-1,2 was precipitated and removed by centrifugation at  $-5^{\circ}\text{C}$ . The ionic strength and protein concentration were 0.05 and 0.04 per cent respectively.

*Antibodies in Fraction II+III.*—In the study of the antibody content of Fraction II+III, and later Fraction II, prepared from normal human plasma by various methods, a large number of immunologists collaborated. Their results for a variety of antibodies have been recorded by Enders (94) at the request of Dr. A. R. Dochez of the committee on Medical Research of the Office of Scientific Research and Development. They found that II+III contained large part of the antibodies reacting with a variety of pathogenic bacteria and their products, viruses, and the isoantigens of human blood groups. Fraction II+III antibodies were four to ten times concentrated over plasma. Fractions II-3 and II-1,2,

were found to contain antibodies reactive with diphtheria toxin, streptococcal erythrogenic toxin, influenza A virus, mumps virus, and the H antigen of *Eberthella typhosa*. These antibodies were concentrated from fifteen to thirty times over pooled normal human plasma. In general, the potency of both fractions was somewhat lower than convalescent serum, but not by a factor of more than four or five. The titer of the antibody reacting with the O typhoid antigen was lower in Fraction II-1,2 than in II-3, and was found in large amounts in Fraction III-1 obtained by procedures other than Method 9. The antibody titers against influenza A seemed to increase slightly following influenza epidemics.

*γ-globulin of Fraction II on measles.*—A study on stability by Enders (94) showed that the titer of the antibodies in Fraction II after prolonged storage and exposure to moderately elevated temperatures was not significantly reduced. Studies on the use of γ-globulin in measles were made by Stokes, Maris & Gellis (95) in Philadelphia and by Ordman, Jennings & Janeway (96) in Boston. Using a 20 per cent solution of Fraction II γ-globulin the former group found that children, five years of age or younger and exposed to measles, required 0.25 cc. to 0.5 cc. for attenuation and 2.0 cc. to 2.5 cc. for protection. Children from six to twelve years of age and exposed required 1 cc. to 1.5 cc. for attenuation and 4.0 cc. to 5.0 cc. for protection. The studies of Stokes, Maris & Gellis using Fraction II + III and Fraction II were made during early stages of measles in fifty-three cases and it was observed that dosages ranging from 5 to 35 cc. resulted in modification of the disease in 40 to 80 per cent of the cases.

The observations made in Philadelphia and Baltimore were confirmed by Ordman, Jennings & Janeway (96) in Boston. The dosages used by these workers were 5 cc. of a 20 per cent Fraction II for children over five years of age, 2.5 cc. for children under five years, and 2.0 cc. for children six months to one year. For studies on protection, injections were made immediately after exposure. For attenuation studies the injections were made six to nine days after exposure. Their first group of studies was on families in which all the children were exposed. Some were uninoculated controls, others were inoculated with the doses given above and were the test group. Of 54 cases studied for protection, 31 were inoculated, and 23 were not inoculated. Of those inoculated, 26 had no measles, 5 mild measles, and none had typical measles. Of the 23 correspond-

ing controls, 1 had no measles, 2 had mild measles, and 20 had typical measles. Of 54 cases studied for attenuation, 31 were inoculated, and 23 were controls. Of the 31 inoculated, 18 had no measles 12 had mild measles, and 1 had typical measles. Of the 23 controls, 2 had no measles, none had mild measles, and 21 had typical measles.

Similar results were obtained by Ordman, Jennings & Janeway during school epidemics and also in private practice cases. They stated that an intramuscular dose of 0.1 cc. per pound of body weight within the first five days after exposure appeared to be adequate for complete protection in a large majority of cases. In order to produce a modification of the disease, about one-quarter of this dose was necessary during the first five days of exposure. To date forty-seven preparations of  $\gamma$ -globulin have been tested and reports of over three thousand injections analyzed by Janeway (97, 98) who confirmed the above dosages but set the time of injection up to within eight days instead of five days. It was recommended that in order to achieve protection or attenuation, the physician should vary the dose rather than the interval of time between exposure and injection. Greenberg, Frant & Rutstein (99) reported on the comparison of  $\gamma$ -globulin and placental globulin in their effectiveness in the prevention and modification of measles. In their study the placental material was not as active and gave nearly fifty times as many reactions as did the  $\gamma$ -globulin. Their immunological results confirmed this as they found that the placental extract was very low in the antibodies that they tested for (99).

*$\gamma$ -globulin of Fraction II in Mumps.*—The  $\gamma$ -globulins in Fraction II have not been concentrated sufficiently to be of value for prophylaxis against mumps although Enders (94) reported that the antibodies against mumps were concentrated in Fraction II as judged by complement fixation. In convalescence from mumps, however, these antibodies were found to be elevated ten- to thirty-fold. Fractionation of mumps convalescent plasma gave  $\gamma$ -globulin that had ten to fifteen times more complement-fixing antibodies than normal human serum  $\gamma$ -globulin. Although its prophylactic value has not been proved, Gellis, McGuinness & Peters (100) demonstrated a lowering in the incidence of orchitis when  $\gamma$ -globulin from mumps convalescent serum was administered on the first day of the disease.

*$\gamma$ -globulin of Fraction II in other conditions.*—Studies on the effect of  $\gamma$ -globulin in chicken pox and infantile diarrhea have given very little, if any, hope of its prophylactic or therapeutic value. Like the antibody to mumps in convalescent serum, the antibodies to scarlet fever are elevated ten- to thirtyfold and this serum has been used in the treatment of disease. The importance of hemolytic streptococcal infections during the war made possible the use of  $\gamma$ -globulin in the treatment. No final conclusions as to its value have been made as yet due to the complexity and nature of the condition. Convalescent plasma has been fractionated with the result that the  $\gamma$ -globulin from this source was four to five times more active than that prepared from normal plasma.

In their studies on poliomyelitis, Kramer (101) and Stokes (102) have prevented the disease in monkeys, cotton-rats, and mice if  $\gamma$ -globulin was injected before the virus. Since the incidence is about one in a thousand susceptibles it would be necessary to immunize passively practically an entire population. Bahlke & Perkins (103) studied the effect of the globulin in large doses in preparalytic cases and found the human antibody does not affect the course of the disease once the symptoms are demonstrated.

*$\gamma$ -globulin of Fraction II in infectious hepatitis.*—The effect of  $\gamma$ -globulin in infectious hepatitis has been studied by Stokes and his collaborators. Stokes & Neefe (104) found that the disease could be either prevented or modified in exposed individuals by the injection of  $\gamma$ -globulin. However, in the reports (105, 106) from the Mediterranean Theater of War the value of  $\gamma$ -globulin in the treatment of hepatitis was difficult to determine. Havens & Paul (107) were able to observe a passive protection by  $\gamma$ -globulin injections during an epidemic in an institution for children, thus confirming similar observations made earlier by Stokes & Neefe (104).

#### FRACTION IV

*Chemical procedure.*—The precipitation of Fraction IV has been accomplished in several ways (40). In Methods 1 to 5 this fraction was separated as one precipitate but contained a considerable amount of denatured material. Method 6 was developed to overcome this. Those proteins, which were precipitated in Fraction IV at pH 5.8 and 40 per cent ethanol, and found to be denatured, were readily precipitated without being demonstrably altered at a pH around 5.1 and 18 per cent ethanol. This observation was taken

into account in the development of Method 6 which included the separation of a fraction, IV-1, under these conditions.

In the earlier methods the supernatant fluid following the precipitation of Fraction II+III was adjusted to 40 per cent ethanol, pH 5.8, ionic strength 0.09, protein concentration 1.06 per cent and temperature  $-5^{\circ}\text{C}$ . All the globulin remaining in solution after precipitation of Fractions II+III was separated as Fraction IV. In Method 6, supernatant II+III material was adjusted to 18 per cent ethanol, pH 5.1, ionic strength 0.09, protein 1.58 per cent, and temperature  $-5^{\circ}\text{C}$ . and Fraction IV-1 precipitated. Although the proteins in this fraction were unchanged, they were denatured easily by high ethanol concentrations. The supernatant fluid, following precipitation, was adjusted to 40 per cent ethanol, pH 5.8, ionic strength 0.09, protein concentration 1.01 per cent, and temperature  $-5^{\circ}\text{C}$ ., and Fraction IV-4 was precipitated. The supernatant material remaining contained very little if any globulin and practically all the albumin that normally occurs in plasma.

*$\alpha$ -lipoprotein and other components in Fraction IV-1.*—Fraction IV-1 and Fraction IV-4 have been studied from both chemical and physiological standpoints by many investigators. The studies, however, began just prior to V. J. Day, and as a result very little concerning either has been published. Fraction IV-1 has been separated into at least three subfractions, one of which contained most of the lipid of the plasma that was combined with  $\alpha$ -globulin. Another subfraction was found to contain the proteins described by Green (108) and Luetscher (109). A third was suggested as having value in the differentiation of true from false Wassermann tests (110).

*Precipitation of hypertensinogen.*—Hypertensinogen or renin activator has been found in the supernatant fluid of Fraction IV-1 (111) but the conditions described (40) for precipitating Fraction IV-4 destroyed it almost completely. Only 10 per cent could be found in Fraction IV-4 and the remainder could not be accounted for. If, however, the pH of the supernatant fluid of IV-1 was lowered to pH 4.7 and the ethanol increased to 25 per cent, hypertensinogen was precipitated quantitatively. Under these conditions practically all the albumin precipitated with the hypertensinogen. Although no method has yet been developed to separate the hypertensinogen from the albumin so precipitated without destroying

the former, such a procedure will unquestionably be found. Meanwhile Fraction IV-4+V prepared in this way might prove a satisfactory source of hypertensinogen.

*Physiological importance of Fraction IV-4.*—Unpublished studies have demonstrated that Fraction IV-4 is a rich source of serum esterase (112) and an iron-binding globulin (113). Methods of subfractionation of Fraction IV-4 have been developed which have concentrated each into a separate fraction. Oncley, Scatchard & Brown (114), who have studied the molecular dimensions and osmotic behavior of the plasma proteins that have thus far been separated, have found a  $\beta$ -globulin with a molecular weight similar to that of albumin and an  $\alpha$ -globulin with a much higher molecular weight.

### FRACTION V

During the war the primary interest in fractionation of plasma was in the albumin that could be used clinically. The by-products could not be salvaged until albumin was well into production, although the original plan called for making available in time all the by-products of plasma. A satisfactory procedure for separating the antibodies, thrombin, fibrinogen, and isohemagglutinins was, however, not developed until much evidence had accumulated, indicating that these substances were being concentrated in those fractions which were removed during the process for giving a final globulin-free albumin.

*Chemical procedure.*—In each of the methods of plasma fractionation as described by Cohn *et al.*, (40) albumin was the last of the proteins removed. The fraction in which it was initially precipitated was known as Fraction V and was even without further purification 95 per cent albumin, 4 per cent  $\alpha$ -globulin, and 1 per cent  $\beta$ -globulin on electrophoretic analysis (Table I). The supernatant fluid remaining after precipitation of IV-4 was filtered through a clarifying pad and adjusted to pH 4.8. The ethanol concentration was kept at 40 per cent as in the precipitation of IV-4. The temperature was maintained at  $-5^{\circ}\text{C}$ ., but the ionic strength and protein concentration were adjusted to 0.11 and 0.75 per cent, respectively. Under these conditions Fraction V precipitated. To insure a purer albumin, Fraction V was reprecipitated by dissolving Fraction V in 10 per cent ethanol, ionic strength 0.01, temperature  $-2^{\circ}\text{C}$ . to  $-3^{\circ}\text{C}$ ., protein concentration 3 per cent,

and pH 4.5 to 5.2. Under these conditions albumin was soluble but the impurities were insoluble. After filtration to remove the insoluble impurities, the ethanol concentration was increased to 40 per cent and the albumin reprecipitated. By electrophoresis this precipitate was 98 to 100 per cent albumin and was the material dried under reduced pressure for storage and subsequent distribution to the armed forces as a compact, concentrated solution for the treatment of shock, edema, and hypoproteinemia.

*Albumin in shock.*—Various laboratories have studied the effect of albumin in man. Janeway *et al.* (115) stated that albumin had at least two known functions. It maintained the colloid osmotic pressure of the blood and played a role in the nutrition of the tissues. The preliminary studies of Stead & Ebert (116) on human subjects in shock showed that albumin increased the circulating blood volume by drawing on the tissue fluids of the patient. Measurements by Scatchard, Batchelder & Brown (117) indicated that each gram of albumin should hold 18 cc. of fluid in the circulation. In conjunction with this group, Heyl, Gibson & Janeway (118) measured the plasma volume increase produced in man by albumin injection following rapid blood depletion by venesection. The average increase was 17.4 cc. per gram one hour after injection. On the basis of these experiments 25 grams of albumin in 100 cc., which represented the osmotic equivalent of 500 cc. of citrated plasma, was taken as the standard dose. The studies of Janeway *et al.* (115) showed that injection of albumin caused a rapid fall in hemoglobin concentration and hematocrit reading. There was no fall in plasma protein concentration as was the case when saline was injected. The effect was well sustained in patients with previously depleted blood volumes, but was transient in those with normal levels.

*Albumin in dehydrated animals.*—The question as to whether injection of a concentrated albumin was either ineffective or harmful to severely dehydrated individuals in shock has been studied in animals and in humans. Studies with bovine albumin injections into animals were reported by Fine, Frank & Seligman (119) who observed dogs in tourniquet shock and found concentrated albumin ineffective unless saline was given by stomach tube. Mahoney, Kingsley & Howland (120, 121), studied the effect of both albumin and plasma in the treatment of severe shock due to intestinal trauma in dogs and found that dilute and normal plasma were more effective than concentrated plasma. Twenty-five per



cent albumin did more than concentrated plasma to restore the circulation, but the animals did not survive. Dunphy & Gibson (122) have observed the effect of both concentrated and dilute bovine albumin solutions in the treatment of severe burns in dogs. The circulation was sustained by the concentrated material but tissue damage was found on microscopic examination. Such was not the case with dilute albumin.

*Albumin in dehydrated patients.*—The first report concerning the administration of albumin in humans was made by Heyl & Janeway (123) who showed that the increase in plasma volume due to the injection of concentrated albumin was not much different in subjects with mild dehydration than in subjects who had been given large quantities of salt and water by mouth. Later a report by Beecher (124) from the Mediterranean theater indicated that 100 cc. of concentrated 25 per cent albumin was only half as effective as 500 cc. of unconcentrated plasma. Additional fluids had not been used and the comparison of equal volumes of 5 per cent (isotonic) albumin and plasma had not been made. A study requested by the Navy and conducted under the auspices of the committee on Medical Research by four teams working on shock and directed by Dr. D. W. Richards, Jr., revealed that although concentrated albumin produced an immediate increase in plasma volume in most shock cases, averaging 10 to 12 cc. per gram of albumin injected, the full increase of 18 cc. per gram was not obtained until additional fluids were administered.

A second question regarding the use of albumin was whether globulin-poor albumin when injected in large amounts may lead to a deficiency in those globulins pertaining to blood coagulation and immunity. This was investigated by Janeway (125) who studied the effect of bovine albumin in dogs and humans. He showed that there was considerable margin of safety before a decrease of the antibacterial or phagocytic powers of the blood could be detected.

To determine the safety of albumin proven sterile, pyrogen free in rabbits and safe in guinea pigs and mice, 1,915 injections were given to six hundred patients (115). No reactions occurred in properly processed materials.

In confirmation of the effect of albumin in shock, several groups of workers (126 to 129) compared albumin injections to whole blood transfusions in twelve cases of traumatic injury. Recovery from shock with increased right auricular pressure, arterial pres-

sure, and cardiac output was observed on albumin treatment. The cardiac output was somewhat greater than that with whole blood and was explained as a compensatory effect to increase the oxygen transport to the tissues in cases where hemo-dilution was observed. It was stated that whole blood should follow albumin because of the anemia that was found in many cases.

*Albumin in hypoproteinemia.*—Evidence for the use of albumin in the treatment of hypoproteinemia has been presented by Jane-way *et al.* (115). The patients studied were divided into groups: (a) those with an inadequate protein intake; (b) those with altered ability to synthesize albumin; (c) those with excessive loss of protein from the body; and (d) a group of miscellaneous cases. Very large amounts of albumin were needed to raise the level of albumin in the serum in patients with chronic protein depletion. The fate of the injected material could not be determined, for only a small portion was found in the blood stream and very little appeared in the urine except in two patients with nephrosis. In all patients with normal kidneys the albumin injected was not found in the urine and it was assumed by the above investigators that the protein was being stored. Repeated albumin injections in patients with cirrhosis of the liver raised the level of serum albumin, decreased the level of serum globulin, and temporarily improved the state of nutrition. A limiting factor in the elevation of the serum protein level by albumin seemed to be the capacity of the circulation to adjust to increases in blood volume.

*Thermal stability of albumin.*—Up to the time when albumin was prepared salt poor, all final containers held 100 cc. of solution containing twenty-five grams of human serum albumin in 0.3 *M* sodium chloride at a pH of 6.8, with merthiolate at a concentration of 1:10,000 or its equivalent, as a preservative (130, 131). The salt concentration of 0.3 *M* was adopted as a result of a study on thermal stability of human albumin by Scatchard *et al.* (132). The stability was greatest when the salt concentration was 0.3 *M*. The optimum pH was found to be around 6.8. The preservative used had very little if any effect on the final stability. A good preparation of albumin could be heated for several months at 50°C. with very little increase in turbidity.

*Low salt albumin.*—As a result of conclusions drawn from the studies of Aldrich *et al.* (133), and by Luetscher (134) who suggested that hypoalbuminemia was difficult to overcome with

plasma because of its high salt concentration, the Harvard laboratory prepared and distributed an albumin solution low in salt (135). The first albumin of this type prepared by this laboratory was a 25 per cent solution of nearly isoelectric albumin in water (17). Such low salt albumins were strongly hypotonic and hemolyzed red cells if injected rapidly without glucose. Furthermore, they were acidic and unstable. The clinical advantages of low salt albumin have been achieved by an extension of the research of Balou *et al.* (136, 137) who studied the influence of nonpolar anions on the thermal stability of albumin using both nephelometric and cloud point methods. They found that in the aliphatic series butyrate was a better stabilizer than acetate, and caprylate was better than butyrate. Other anions such as phenylacetate and mandelate were also extremely effective. The length of time during which serum albumin could be subjected to high temperatures was increased from four to ten times when small concentrations of each of these substances were substituted for the 0.3 *M* sodium chloride. The sodium salts of acetyl phenylalanine (138) and acetyl tryptophane (139) were recently suggested as being amino acid derivatives and therefore more nearly physiological. They were studied and found to be very effective in increasing the thermal stability of albumin.

Brand, Kassell & Saidel (140) analyzed crystalline human albumin and found but 0.9 per cent tryptophane. Hegsted, Hay & Stare (141) found that for normal growth in rats albumin had to be fortified with both tryptophane and isoleucine. Hegsted, McKibbin & Stare (142) showed further that plasma is low in isoleucine but not in tryptophane. Albumin stabilized with 0.04 *M* acetyl tryptophane or mandelate was heated for ten hours at 60°C. and found to be more stable than that with 0.3 *M* sodium chloride (135). A mixture of 0.02 *M* acetyl tryptophane and 0.02 *M* caprylate permitted heating at 63°C. The new standard albumin solution is one which is a 25 per cent solution at pH 6.8 with 0.04 *M* sodium acetyl tryptophanate without a mercurial bacteriostatic agent, but pasteurized ten hours at 60°C. In such a preparation the amount of sodium ion was not over one-third that of previous solutions. The use of salt-poor albumin in nephrosis was studied by Thorn *et al.* (143). Material was injected intravenously for periods varying from one to thirty days at a dosage of fifty grams a day and a rate of ten grams an hour to seven patients in several stages of chronic nephritis who were maintained on a diet adequate in calories, con-

taining 80 to 125 grams of protein daily and low in sodium chloride. Salt-poor albumin injections increased the serum albumin level and induced a positive nitrogen balance in individuals with edema, low serum proteins, and with little hypertension and nitrogen retention. In the severe nephrotic state with marked edema the protein was diuretic during the administration but not afterward. Further studies perhaps will give a clearer picture of the effect of albumin and its fate in the body.

Albumin was found to have a specific role in liver disease in that elevation of the serum protein was accomplished by Thorn, Armstrong & Davenport (144) in individuals suffering from cirrhosis of the liver. A similar observation was made by other investigators (145) in surgical patients with low serum proteins. Janeway (98) has treated infants and children having a low serum protein with albumin containing sodium chloride but nothing has been reported on the use of low salt albumin.

*Albumin as a stabilizing and combining protein.*—Albumin has been the source of study not only in the clinic but also in the laboratory. Cameron & Diamond (146) and Diamond & Denton (147) have shown that albumin in high concentration enables agglutination to occur with the anti-Rh globulin fractions high in "blocking" antibodies. It also stabilized the anti-Rh globulin. Dubos (148) showed that serum albumin, either bovine or human, when added to a suitable medium, would support growth of tubercle bacilli, although little or no growth took place in the absence of albumin. The effect of protein here was traced to its action in removing certain growth inhibitors which belonged to the class of such unsaturated fatty acids as oleic acid. Other proteins were studied but albumin was found to display the highest specific capacity to bind oleic acid. Heat denaturation of the albumin was found to abolish this specific property. Kendall (149) found considerable amounts of fatty acids in human serum albumin crystallized from ammonium sulfate. Boyer *et al.* (137, 150, 151) studied the effect of various substances on the stability of albumin and concluded that the basic requisite structure for stabilizing albumin is an anion with a nonpolar group attached. Further, they stated that the action of the fatty acids is due to their combination with certain groups or areas of the albumin molecule, probably a combination of the anion with both the positive groups and the nonpolar portions of the albumin. Further investigations are necessary to learn the nature of the protein groups involved.

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DEPARTMENT OF PHYSICAL CHEMISTRY, HARVARD MEDICAL SCHOOL,  
AND MASSACHUSETTS DEPARTMENT OF PUBLIC HEALTH  
BOSTON, MASSACHUSETTS

## BLOOD GAS TRANSPORT<sup>1</sup>

BY D. B. DILL AND W. H. FORBES

*Laboratory of Industrial Physiology, Graduate School of Business  
Administration, Harvard University, Boston 63, Massachusetts*

*Introduction.*—It is the purpose of this review to bring the reader up to date on the subject of transport of gases by the blood, including particularly oxygen, carbon dioxide, carbon monoxide, and nitrogen. Since this specific subject has not been covered before in this Review, it has been necessary to go back on occasions several years to pick up loose ends. New and improved methods will be surveyed followed by references to standard values for blood components involved in gas transport, new material on carbonic anhydrase, hemoglobin and related pigments, and pertinent research on high altitudes, exercise, and comparative physiology.

*Methods: Gases in blood.*—There are three main types of analyses for the gases commonly found in blood (oxygen, carbon dioxide, nitrogen and carbon monoxide): the first measures the quantity of these gases present, usually by extraction of the gases in vacuo with ferricyanide, followed by analysis of the bubble; the second measures the tension of the gases either by electrodes inserted into the blood or tissues or by the introduction of a small bubble which rapidly reaches equilibrium with its surroundings and is then withdrawn and analyzed; the third (which is applicable only to oxygen and carbon monoxide) measures the percentage saturation of the hemoglobin by spectroscopy, commonly employing a photoelectric colorimeter and filters.

The micro methods of Scholander & Roughton (1, 2, 3, 4) are of the first type and are outstanding for their combination of reasonable accuracy with economy of time and apparatus. On a single sample of 40 c.mm. obtained from a finger prick the oxygen, nitrogen, and carbon monoxide can be determined with an accuracy of  $\pm 0.2$  volumes per cent in ten to fifteen minutes. A similar method for carbon dioxide (4) requires a separate sample and is less accurate ( $\pm 1.0$  volumes per cent). Adaptations of their methods have been made for special purposes, e.g., determining small amounts of carboxyhemoglobin, and often great accuracy ( $\pm 0.02$  volumes per cent) can be attained (5). An unusual method in which

<sup>1</sup> This review covers the period from 1942 to 1946.

the oxygen forms a colored iron salt without ever being extracted from the liquid phase gives an accuracy of 0.5 volumes per cent on 0.1 cc. of blood (6). The Barcroft apparatus is useful when it is desirable to run several determinations simultaneously and agrees well with the Van Slyke if proper precautions are taken (7). The Van Slyke method for carbon monoxide in the blood has been compared with a titration method using palladous chloride (8, 9) [which can be used even with coagulated blood (10)] and a colorimetric method (11). There is good agreement at low values but the titrimetric method is 5 per cent low when the carboxyhemoglobin exceeds 20 per cent.

The second type of analysis, the determination of gas tension by the use of platinum electrodes in tissues (12, 13) or with the dropping mercury electrode in body fluids (14) will give accurate results in skilled hands. These methods probably are not suitable for routine determinations. Equilibration and analysis of a small bubble by a modification of Scholander's techniques (1, 2, 4) is also a satisfactory method for determining the tension of gases in liquids to within  $\pm 3$  mm. (15, 16).

The third type of analysis, the determination of percentage saturation by spectroscopic methods, was widely used during the war in aviation studies particularly in the form of the oximeter, a small photoelectric colorimeter that can be attached to the ear (17). This instrument reads directly in arterial saturation and is accurate to  $\pm 5$  per cent or better with suitable precautions (18). More accurate results can be obtained by arterial puncture and immediate observations of thin films of blood coming directly from the needle into special very thin vessels (19, 20).

A number of simple methods for use with samples of blood *in vitro* have been described both in this country (21) and abroad (22) for measuring both oxyhemoglobin and carboxyhemoglobin using the Pulfrich step photometer or similar instruments (23 to 27).

Dissociation curves may be obtained on small quantities of blood with the reversion spectroscope (28) or by following the fall in oxygen with time and fermentation using both a spectroscope and a dropping mercury electrode (29).

*Methods: Gases in inspired or expired air.*—The early methods of gas analysis were chemical, but the last few years have seen the development of rapid and accurate methods depending on physical properties. The paramagnetic property of oxygen has been used in

an instrument (30) which can follow changing tensions of this gas with a lag of about one second and with an accuracy of 0.5 per cent of its range. It can be designed to work over various ranges of physiological interest. A nitrogen meter depending upon the characteristic discharge glow of this gas in an evacuated tube will follow even more rapid variations (of the order of 0.02 second) with a 1 per cent accuracy (31).

A more conventional development is an extremely simple method for determining nitrogen, used for detecting leaks in aviators' oxygen masks (32). An improved method for analyzing respiratory gas samples of 0.2 cc. or less of gas with great accuracy employs a micrometer plunger displacing mercury in a small vessel (33).

Several delicate methods have been developed for measuring small quantities of carbon monoxide. One of these depends on blood to select and concentrate the carbon monoxide from the gas sample (34); another, on the whole easier, depends on the color change of salts of palladium and molybdenum (35); a third, on heat of reaction of carbon monoxide with hopcalite.<sup>1</sup>

*Standard values.*—The transport of gases by the blood depends on two primary factors: hemoglobin for oxygen and alkaline reserve for carbon dioxide. Secondary factors which are interrelated and depend on hemoglobin and alkaline reserve are the tensions of oxygen and of carbon dioxide and the pH.

Studies of hemoglobin concentrations in England during the war (36) give the first comprehensive body of information covering all age groups and both sexes. Values found, using the Haldane-Gower hemoglobinometer, were 102.4 per cent for unmarried men and 102.1 per cent for married men. Figures for women ranged from 90.8 per cent for women under fifty with children to 94.3 per cent for single women and married women without children. These correspond in terms of oxygen combining capacity approximately to 19 volumes per cent for men and from 16.8 to 17.5 for women. In this country the "textbook" average value for men is 20.7 volumes per cent or 112 per cent on the Haldane scale. The reason for this difference between countries may be real and related to nutritional status, or it may be that comparable samples and statisti-

<sup>1</sup> This principle has been employed in the well-known "continuous carbon monoxide indicator" made by the Mine Safety Appliances Co. of Pittsburgh. New models of this instrument are greatly improved in performance.

cally valid results have not been obtained, particularly in this country. Technical error must always be considered; careful analysis of the observers' reliability is contained in the British report.

Values for hemoglobin concentration in the blood of 4,550 college women has been compiled in colleges and universities of six north-central states (37). In the entire series 72 per cent of the hemoglobin values fell between 12.2 and 14.6 gm. per cent; the extreme range was from 8.5 to 17.5. The state averages varied from 13.0 in 292 women in Minnesota to 13.7 gm. per cent in 225 women in Wisconsin with a grand average of 13.4, corresponding to 18.1 volumes per cent or 97.8 per cent on the Haldane scale if one assumes 1.34 cc. of oxygen combines with 1 gm. of hemoglobin. The authors conclude that "normal standards for hemoglobin concentration . . . must be interpreted as a range of values . . . wider . . . than recorded by earlier studies."

Such a wide range of values raises the question as to definitions of normals; in this connection reference is made to three studies of dog blood. Mulligan (38) showed that hemoglobin in sixty normal dogs studied at Denver (altitude one mile) was 14.5 gm. per cent as compared with a value of 13.5 reported previously in New Orleans by Mayerson (39). One would accept this as an altitude effect were it not for the demonstration by Smith (40) that in ten mongrel pups at sea level, maintained on a diet rich in B vitamins, average hemoglobin increased from 10.5 gm. per cent at eight to twelve weeks to 18.8 fifteen weeks later when the adult plateau was reached. This observation points to the need of "standard" dogs for sound experimentation.

Evidence for a diurnal rhythm in hemoglobin concentration reported by McCarthy & Van Slyke (41) has been reviewed by Mole (42). Experimental observations by Brown & Goodall (43) on hemoglobin concentration in blood samples drawn on each of four to five consecutive days gave a maximum individual range of 2.05 gm. per cent and an average maximum of 0.93. Diurnal values in ten subjects, based on various samples drawn at three-hour intervals from 9 a.m. to 6 p.m. showed an average maximum range of 0.54 gm. Thus it appears important for "standard" samples to be drawn at an agreed time and under constant conditions. Usually it is best to have the subjects resting, reclining, and without breakfast.

The quantity of active hemoglobin is about 3 per cent less than the total pigment. The basis for this conclusion is reviewed in the



section on *Hemoglobin* as is also the evidence for the arterial oxygen tension being approximately 100 mm. Hg. These developments previously reviewed at greater length (44, 45) have been challenged (46, 47). According to these authors the gradient from alveoli to blood consists of two factors—a small one across the alveolar wall and a larger one due to admixture with blood which has not passed through adequately ventilated alveoli. The total gradient is about 9 mm. in rest and double that in exercise. These authors believe that samples of alveolar air as ordinarily drawn are not representative and propose an indirect method, employing the following equation:

$$\text{Alveolar } pO_2 = \text{tracheal } pO_2 - \frac{\text{arterial } pCO_2}{\text{expired air R.Q.}}$$

In any case there is no doubt that when arterial blood of man at rest (breathing air at sea level) is found to have less than 95 per cent of oxyhemoglobin in total pigment (48, 49) four likely interpretations should be considered—technical errors, increased proportion of inactive pigment, abnormal diffusion from lungs to blood or other pathological conditions, such as patent foramen ovale.

The carbon dioxide combining capacity, the carbon dioxide content and tension, and the pH are interdependent and, in health, are controlled within narrow limits in rest but vary widely in exercise and in many diseases. One or more of these interrelations are commonly dealt with under the title of acid-base balance, a subject reviewed by Sendroy in 1938 (50). Since then several pertinent articles have appeared such as that of d'Elseaux and associates (51) which revealed a narrower range in pH than previously reported. The sensitivity of the method was  $\pm 0.003$ . The range in the eighteen normal subjects was 0.033 around a mean of 7.42. Also in a series of fifty normal subjects, Gibbs and associates (52) found an average arterial pH of 7.424 with an extreme range from 7.374 to 7.455 or 0.081 in log units. These small ranges imply correspondingly narrow ranges in carbon dioxide content and tension or at least in the ratio of one to the other. In the latter series arterial carbon dioxide content varied between 44.6 and 50.2 volumes per cent and the carbon dioxide tension between 36.2 and 44.9. The logarithms of these differ by 0.052 in the case of content and 0.092 in the case of tension.

Arterial oxygen and hemoglobin are somewhat more stable than

arterial carbon dioxide and pH. These latter variables are sensitive, for example, to hyperventilation and to exercise. This literature has been reviewed by Rapoport and associates (53) who found in their own experiments that voluntary hyperventilation could lower arterial carbon dioxide by 12 volumes per cent and raise the pH from a range of 7.44 to 7.51 to an average of 7.71 (maximum 7.75). There were dependent changes in electrolytes of plasma and red cells. In view of the foregoing results it is likely that when great variations are found in any of these components in normal arterial blood of man at rest they can be explained by disease, by emotional disturbance of the subjects, or by technical errors.

When venous blood is analyzed one expects greater variability than is found in arterial, particularly if different veins are used and if external temperature varies. Even when these conditions are controlled, oxygen saturation in blood from the arm veins may vary from 25 to 85 per cent according to measurements made on fifty-nine men and twenty-four women (54). In twenty-one men the range for femoral venous blood was 38.4 to 91 per cent. It must be assumed that the observed variations on oxygen saturation depend on variable flow through the extremities rather than variable oxygen consumption. Keys did not record determinations of pH but assuming an R.Q. of 0.8 it is simple to estimate the range in pH between his extreme venous oxygen saturations; using the Henderson nomogram for normal human blood (55), this amounts to 0.05—alkaline reserve being constant.

The stability of oxygen saturation of jugular blood was proved by Gibbs and associates (52). In fifty subjects percentage oxygen saturation varied from 55.3 to 70.7 and pH from 7.321 to 7.397. This points to extraordinary control of the acid-base balance of the brain, more fully borne out by the work of Nims and associates (56) showing that this protection depends on regulation of cerebral blood flow. In hyperventilation the cerebral arterio-venous differences (both oxygen and carbon dioxide) decreased and in breathing air containing carbon dioxide they both increased. The ratio of carbon dioxide transport to oxygen transport was approximately two until the diminishing flow reduced venous oxygen saturation to about 30 per cent. At this point the mechanism protecting the acid-base equilibrium was overridden by the demand for oxygen.

The most notable technical advance in our knowledge of "standard" values has come from application of the catheteriza-

tion technique to obtain samples of mixed venous blood (57, 58). The average arteriovenous oxygen difference in thirteen normal males at rest was 4.5 volumes per cent and the range, 3.5 to 6.0. With an arterial saturation of 97 per cent and a total hemoglobin of 19.0 volumes per cent, expressed in oxygen combining capacity, the venous saturation would be 73 per cent. This represents a cardiac output about one quarter greater than found by the acetylene and ethyl iodide methods as developed and applied in this country but compares closely with ballistocardiographic results (59).

In contrast with all the foregoing evidence for the stability of acid-base balance even of venous blood, there are many reports of wide variations in acid-base balance in laboratory animals, for example cats (60) with a reported venous pH range of 7.31 to 7.54. Both dog and man show wide ranges in venous pH according to Berg and associates (61). The weekly averages of venous pH values throughout a year varied from 7.32 to 7.68 in dogs and from 7.28 to 7.68 in man. The pH values found correlated with temperature and atmospheric pressure, the latter correlation being the higher. Such a wide range in venous pH cannot be explained in the light of our knowledge of the resistance of the organism even to major climatic and weather changes.

Miscellaneous observations in this field include observations of alveolar, and by inference arterial, carbon dioxide tension of males and females showing that the sex difference emerges at age thirteen (62), and continuous records of blood and urine pH during alteration of lung ventilation in anesthetized dogs (63).

*Carbonic anhydrase.*—The work on carbonic anhydrase up to the fall of 1943 has been covered by Roughton's thorough discussion and synthesis in a Harvey Lecture (64). The work has continued along chemical lines with emphasis on activating or inhibiting substances some of which are found in the tissues. Bakker (65) has shown that oxidation or reduction of carbonic anhydrase reduces its activity. He also finds that there is a substance commonly found in serum or tissue extracts which strongly activates the enzyme. This substance may be adsorbed on Norit. He tried other substances which had been reported to be activators and found them to be without effect. Leiner (66) also reports activators, as well as inhibitors, from the plasma of various species. Human or rabbit plasma, heated or unheated, activates the enzyme while hog, horse,

cat or sheep plasma act as inhibitors unless they have been heated, in which case they act as activators. Though it is clear that under a given set of experimental conditions the addition of a substance such as rabbit plasma may increase the rate at which carbon dioxide is given off, it is essential to control many factors before one can be sure that these are cases of true activation (67). Carbonic anhydrase is unstable especially when in dilute solutions and in the absence of proteins. Consequently an increase in apparent activity may be merely a prevention of breakdown. Another possibility is that these "activating" substances may suppress inhibitors.

The existence and nature of inhibitors is undoubted and has been very helpful in the study of the enzyme. The strongest specific inhibitor is thiophene-2-sulfonamide (68) which is six times as effective as sulfonilamide at 0°C. and probably forty times as effective at body temperature. This substance is a good tool for studying the physiological uses of the enzyme. High pressures of oxygen do not affect carbonic anhydrase (69) as it contains no sulfhydryl group (70). Progress with the chemistry and purification of this substance (71, 72) has been greater than progress with the physiological variables (73). Its activity is decreased with increasing pH produced by hyperventilation, altitude, or injection of sodium bicarbonate, and increased when the pH is lowered. Ingesting 500 mg. of ascorbic acid prevents the increase in pH and accompanying decrease in the activity of carbonic anhydrase. Chenykaeva (74) found with increasing degrees of anoxia an initial increase in the activity of carbonic anhydrase followed by a decrease at moderate anoxia with an uncertain increase in extreme anoxia.

The distribution of carbonic anhydrase within the body of man and animals presents a complex picture (75, 76, 77) with such large unexplained irregularities that only rough generalizations can be made. Man has about the same amount in his blood as a pig or guinea pig has, but this is half the blood level in rats, cats or cattle and double the level in calves or chickens. The amount in muscle varies from nothing to one-fourth the blood level; in nervous tissue and liver it may rise to one-tenth of the blood level while adrenal glands and embryos appear to have none. In man, the occipital region of the brain is about 30 per cent richer than the frontal lobes, a difference which is not found in dogs. In chickens (78) the concentration diminishes from the brain down the cord.

*Hemoglobin.*—Under normal circumstances a small but signifi-

cant fraction of the hemoglobin, as determined colorimetrically, may be present in the form of a related substance which, however, is incapable of carrying oxygen (79, 80). This finding has been questioned (81) but has wide support. In most cases this substance appears to be methemoglobin, but the possibility of other pigments being present also has not been ruled out. As yet there does not seem to be any indication as to what causes this "ferrihemoglobinemia" [except in cases where drugs have been given (82)]. Nor has the usefulness, if any, of this condition been demonstrated in normal circumstances though in cyanide poisoning methemoglobin is helpful (83). Methemoglobin produced in dogs by the intravenous injection of sodium nitrite or *o*- or *p*-aminophenol disappeared at the rate of 11 per cent per hour. Glucose did not affect the rate but methylene blue hastened the disappearance (82).

The early work of Barcroft and his associates on comparisons of fetal and maternal blood has been followed by demonstrations (84, 85) that there are at least two molecular species which differ in solubility, in electrophoretic mobility, and in sedimentation constants. In humans both species are generally present in the fetus as well as in the adult but the proportions are markedly different.

The crystallography of hemoglobin has attracted the attention of a long line of scientists who hoped to obtain and have obtained therefrom information concerning its structure, species specificity, and degree of purity. Human hemoglobin is peculiarly difficult to crystallize and though it has been done the crystals have been small and unsatisfactory for study of their structure. Drabkin (86) has finally obtained excellent crystals of a suitable size and perfection to admit of detailed study and exact description. They are pyramidal in contrast to the various shapes previously described for human hemoglobin. These crystals on drying slightly change partially over to methemoglobin as do the crystals from other species but this change is not accompanied by any profound alteration in the structure, for on treatment with sodium hyposulfite it returns to hemoglobin. This change to methemoglobin may be prevented almost entirely by drying under vacuum at low temperatures. On exposure to air there is a rapid change to methemoglobin (87).

*Oxygen at increased pressure.*—Arterial blood usually carries about 0.25 volumes per cent of dissolved oxygen; nearly two volumes per cent is carried when breathing an atmosphere of pure

oxygen. By further increase in pressure the entire transport of oxygen can be accomplished by dissolved oxygen, leaving hemoglobin fully saturated as blood returns to the lungs. The Bohr effect is not used; carbon dioxide is transported by buffer action alone, producing for a given oxygen transport increments in pH and carbon dioxide tension about double their usual value. These increases are reflected chiefly in venous blood and in tissues (88, 89). Blood gas changes also occur incidental to the decreased cardiac output, which amounts to 15 per cent according to the ballistocardiographic method as used by Whitehorn and associates (90). Arterial carbon dioxide and pH remain unchanged for twenty-four hours while breathing pure oxygen (91). In longer exposures thickened alveolar epithelium may interfere with diffusion of gases; death may result from anoxia as first shown by Binger and associates (92).

*Carbon monoxide.*—One subject which has been greatly clarified by recent work is the behavior of carbon monoxide in the lungs and in the blood stream. Roughton and his associates (93 to 98), Lilienthal and his associates (99, 100, 101) and others (102, 103, 104) have developed the following picture upon which they are substantially in agreement. The combination of carbon monoxide with hemoglobin is so rapid that during the early part of an exposure all of this gas that reaches the blood is immediately combined with hemoglobin. A fraction (10 to 20 per cent) fails to diffuse through into the blood because of the very small pressure of this gas in the alveoli. This fraction is exhaled, together with that part which is in the "physiological dead space," and never reaches the alveoli. In rest, therefore, the concentration in the expired air is about one-half the concentration in the inspired air. In work the increased "physiological dead space" and especially the reduced time allowed for diffusion result in the expired air containing about 60 per cent of the amount inspired. As the exposure progresses and the saturation rises to over 30 or 40 per cent of its equilibrium value the pressure of carbon monoxide gradually rises in the blood and alveoli and diminishes the rate of uptake. Very high tensions of oxygen in the alveoli will also diminish somewhat the rate of uptake, as well as greatly lower the equilibrium value. This follows from Roughton's demonstration that in the presence of oxygen the rate of the reaction  $\text{CO} + \text{Hb} \rightarrow \text{COHb}$  is inversely proportional to the oxygen tension. However, breathing even 99 per cent oxygen at sea level

will only diminish the rate by 23 per cent in rest or 38 per cent in heavy work. As mentioned above the increased ventilation in heavy work at normal oxygen pressures also causes a divergence from the linear relationship of ventilation to uptake because of the shorter time which rapid breathing allows for diffusion. The divergence is pronounced at thirty liters per minute but with ventilations below twelve liters it is not significant.

It follows from these concepts that though high oxygen pressure will diminish the initial rate of absorption of carbon monoxide, low oxygen pressure cannot increase it (since it is already maximal), and this has been shown to be true in experiments at altitude. Provided the carbon monoxide pressure in the trachea and the ventilation remain constant, altitude has no effect on the initial rate. It will, however, greatly affect the rate later in the exposure as the oxygen pressure determines the final equilibrium value and consequently the time at which the back pressure of carbon monoxide in the blood begins to be important.

From the practical point of view the initial rate is important as it is the maximum rate, and hence calculations based upon it may be used to set safe limits of exposure, using the figures 50 per cent in rest and 40 per cent in work as the fraction of the inspired carbon monoxide which will combine with the blood in a given time.

The elimination of carbon monoxide has also been studied and again the observers are in good agreement. There are differences between individuals but on the average it takes about 250 minutes for the level of carboxyhemoglobin in blood to fall to one-half its original value in an individual who is breathing air at sea level (95). To express it another way, at the end of each hour's breathing the blood will have lost 15 per cent of the carbon monoxide which it had at the beginning of that hour. As ventilation increases due to work or inhalation of carbon dioxide the elimination increases also but less than proportionately (103). Breathing pure oxygen will increase the rate of elimination about five- to sixfold so that the "half time" for the blood is reduced from 250 to 40 or 50 minutes, or with 7 per cent carbon dioxide in oxygen, to about 30 minutes.

Under most conditions the limiting factor in the elimination of carbon monoxide, as well as in its uptake, appears to be the slow rate of diffusion of gas at the very low pressure gradients en-



countered while breathing air at sea level. When oxygen is substituted for air the carbon monoxide pressure in the blood rises and the rate of dissociation of carboxyhemoglobin plays about a 30 per cent role in determining the overall rate. With 7 per cent carbon dioxide added to the oxygen the rate of dissociation may even be the predominant factor controlling the rate of elimination.

The carbon monoxide lost from the blood however is not in all cases at once lost from the body. If taken in rapidly as the result of a short exposure to rather high concentration only 60 per cent of the carbon monoxide which disappears from the blood during the first hour of recovery can be found in the expired air. Roughton by using radioactive carbon and Geiger counters has shown that it is not oxidized and that there appears to be an accumulation of the radioactive carbon monoxide in the region of the liver. Eventually i.e., after four hours of breathing oxygen, the full amount of inspired carbon monoxide reappears in the expired air. The mechanism of the phenomenon is not clear.

*Nitrogen.*—Nitrogen is carried by the blood in simple solution ordinarily in equilibrium with other tissues and with atmospheric nitrogen. Oxygen breathing or a change in barometric pressure establishes a new equilibrium after several hours; if a large decrease in pressure occurs rapidly, either in ascent to great altitude or in return to sea level after a dive, bubbles of nitrogen may form in the tissues, a phenomenon variously called, *aeroembolism*, *aeroemphysema*, *bends*, and *decompression illness*.

The transport of nitrogen by the blood has been studied by Scholander and Edwards (105), applying the micro method of Scholander (33). Breathing oxygen clears finger blood and saliva with equal rapidity, blood nitrogen dropping from 1.02 volumes per cent to one-tenth or one-fifth of this value in ten minutes and to 0.06 volumes per cent after one hour. Whiteley & McElroy (106) found that in exercising cats breathing pure oxygen, blood nitrogen dropped to 0.08 volumes per cent within one minute; in resting cats it dropped to 0.10 per cent in about ten minutes. In view of the rapidity with which nitrogen is cleared from blood it is difficult to interpret the slow clearance of radon injected intravenously (107).

*Altitude.*—War investigation in aviation medicine has been accelerated by the invaluable bibliographies of Fulton and associates (108, 109) and by such reviews as those of Behnke & Stephenson,

Matthews, Gemmill, and Monge (110, 111, 112, 113). Monge, Hurtado, and their associates have made Lima, Peru, America's chief center of research in mountain physiology with its National Institute of Andean Biology. This institute has well-equipped laboratories, one in the medical school at Lima and another two hours' distant in the mountains at Morococha, 14,890 feet. A recent study in this institute (114) indicates that the affinity of blood for oxygen is slightly less in both newcomers and residents at altitudes of about 15,000 feet than at sea level.

Man requires weeks if not months to acquire full acclimatization to high altitude. Given time, even at as low an altitude as five thousand to six thousand feet, acclimatization occurs, as is indicated by hematological studies at Witwatersrand, South Africa, altitude 5,740 feet (115). Red cell counts averaged 5.6 million in thirty normal European males and 5.0 in thirty females and hemoglobin was correspondingly high. While man has climbed nearly to 29,000 feet he has never established year-round residence above 17,500 feet. Yet guinea pigs are reported to have become acclimatized to altitudes of 23,000 to 30,000 feet after daily exposures of six hours per day, six days per week for six weeks (116). One should not forget that acclimatization, if complete, implies capacity not merely for six hours' survival but also for maintenance, growth, and reproduction. In long and frequent exposures to altitudes of about 10,000 feet, such as may be experienced by commercial airline crews, increases in hemoglobin have been reported (117, 118). Such a polycythemia may depend on dehydration as judged by an investigation of the early stages of adaptation to simulated altitude in a pressure chamber. Asmussen & Nielsen (119) found that after six hours in a pressure chamber at a barometric pressure of about 450 mm. Hg, red cell count increased significantly partly because of water loss from the body and partly because of movement of water from the blood to the tissues, related perhaps to increased capillary blood pressure.

When man is exposed suddenly to altitudes above 10,000 feet without oxygen, arterial oxygen content and tension decrease, eventually respiratory volume increases, arterial carbon dioxide content and tension then decrease, pH increases, and there is a concomitant rise in arterial oxygen. The extent and time-course of these changes depend on characteristics of the respiratory center, on conditions in the lungs and on cardiac output (120, 121).

Occasional individuals are hyperreactive to oxygen lack, particularly when this is coupled with the emotional stimulus of high altitude flight (122). In the same connection Houston (123) has demonstrated that in subjects breathing 10.5 per cent oxygen at sea level, an increase of one-half in ventilation raised arterial oxygen content by one-tenth to one-fifth, while arterial oxygen tension varied in a linear fashion with ventilation. Small increases in ventilation improved oxygenation without symptoms of acapnia. There was improved mixing of tracheal and alveolar air, partial replacement of carbon dioxide with oxygen, and temporary elevation of the respiratory quotient while the subjects were undergoing adaptation to this atmosphere. A degree of hyperventilation is essential for the most successful response to altitudes of 10,000 feet or above when oxygen is not supplied. The questions are: (a) What tension of carbon dioxide is critical? (b) What percentage of men engaged in high altitude flights carry hyperventilation to extremes? The first question has been answered (124): performance was little impaired until carbon dioxide tension fell below 25 mm. Hg. In 167 man-flights Lawrence and associates (125) report two cases of hyperventilation as judged by clinical examination, unconfirmed by blood studies. Under combat conditions (126) clearcut cases of hyperventilation were rare. Blood samples taken at high altitude on a quiet day had a normal alkaline reserve. On another day in a dangerous situation alkaline reserve was high rather than low, indicating hypoventilation. In commercial aircraft hyperventilation by passengers is rarely seen (127).

Provided oxygen supply is adequate and painful experiences absent, respiratory volumes, total oxygen consumption, and oxygen and carbon dioxide of arterial blood remain unchanged up to an altitude of about 35,000 feet, above which the oxygen pressure in lungs and blood falls below its value at sea level. There is a lag in the respiratory response just as there is when breathing air at 10,000 feet and above, but eventually respiratory volume increases and blood carbon dioxide decreases. At 44,000 feet, breathing pure oxygen, arterial oxygen saturation is about 70 per cent, a state reached at about 18,000 feet breathing air (128, 129). A bold approach to such questions has been opened by the application of the technique of jugular puncture coupled with observations of cerebral blood flow and determination of oxygen and carbon dioxide transport and metabolism. This has been employed, for ex-

ample, to evaluate quantitatively the effects of hyperventilation, of breathing pure oxygen and mixtures of air and nitrogen (130).

Above 40,000 feet there is the possibility of improving oxygenation of the blood by "pressure breathing," as described by Gagge and associates (131) who have been chiefly responsible for research, development, and application in this field for the Army Air Forces. With suitable equipment the airman can breathe comfortably and safely at a positive pressure of eight inches of water (16 mm. Hg) which raises his blood oxygen enough to increase his effective ceiling by four thousand to five thousand feet. Basic research, sponsored by the Committee on Medical Research, Office of Scientific Research and Development, has provided invaluable guides in this development (132, 133, 134).

The foregoing experiments were concerned with oxygen saturations which can be tolerated by most healthy men for a long time, even indefinitely if there is adequate opportunity for acclimatization. At lower oxygen tensions the hazard becomes greater with loss of consciousness apt to occur if atmospheric air is breathed above 20,000 feet (135, 136, 137).

Many studies have been carried out on the survival times of animals at very low ranges of oxygen (138, 139, 140, 141). In one such study (142) the physiological state of rats acclimatized at 5,280 feet was compared with their state after a few minutes exposure to an altitude of 40,000 feet. Arterial oxygen dropped from 18.6 to 4.2 volumes per cent and carbon dioxide from 37.2 to 17.8 volumes per cent. Corresponding venous figures were 9.4 to 1.2 and 47.3 to 27.6. The blood respiratory quotient at the lower altitude, 1.19, indicated an unstable respiratory state. The rats were in a precarious condition after five minutes at 40,000 feet, as evidenced by extreme hyperventilation together with a 50 per cent decline of oxygen uptake by tissues.

The possibility of improving altitude tolerance by varying the diet, by administering drugs, or by other means, has intrigued many workers. King and associates (143) have demonstrated significant gains in altitude tolerance when subjects were fed high carbohydrate diets over the tolerance found after a high protein meal. The improvement has been attributed to an increased respiratory quotient and a lower oxygen requirement, making for more favorable supply of oxygen by the blood to the tissues. This is supported by others (144, 145). D'Angelo (146) agrees

with the effects of carbohydrate feeding on anoxia with the reservation that later hypoglycemic reactions may occur that affect adversely well-being and efficiency in flight. Reduction in food intake gave cats an improved altitude tolerance (147). Whether this depended on a diminished oxygen requirement, as it does in small animals exposed to low temperatures (148), was not determined nor was attention given to the finding that salt deprivation reduces oxygen consumption (149).

Various drugs have been proposed for increasing anoxia tolerance in man. Ammonium chloride according to one report (150) is effective if enteric coated tablets are used, 5 gm. after each meal. A novel method of increasing the resistance to anoxia has been suggested by the finding that injections of cytochrome C, prepared from beef heart, increased the tissue uptake of oxygen in anoxic dogs as indicated by an increased arterio-venous oxygen difference (151).

Davidson (152) followed up the report that radiating blood with a mercury vapor lamp increased oxygen saturation and got positive results. It is difficult to interpret such a finding unless one assumes that in the healthy man there is a considerable pressure gradient of oxygen from alveoli to arterial blood.

The combined action of carbon monoxide and of diminished oxygen is occasionally encountered in aircraft. The rate of carbon monoxide uptake and the amount combined with hemoglobin at equilibrium have been defined by Lilienthal and associates (99, 100). The degree of impairment at various altitudes and carbon monoxide concentrations has also been described (97, 101).

Apparent discrepancy between conclusions as to anoxia tolerance depends in part on definition of terms. Resistance of man is usually measured in terms of functional adequacy of the central nervous system while animal tolerance commonly involves determining the zone that separates life from death. Pertinent comment on researches in this area has been made by Gemmill (113).

*Exercise.*—Knowledge of gas transport by the blood during exercise may be limited by the problem of obtaining the desired blood samples. Arterial blood can be drawn easily from the radial or brachial arteries in such work as riding the bicycle ergometer, or even in grade walking, but not when a man has reached his highest oxygen consumption and is accumulating an oxygen debt. With the perfection of micro methods of blood analysis, some have

turned to the use of finger or ear-lobe blood in lieu of arterial (153, 154). These authors established values for the composition of ear-lobe blood by applying radiant heat, at various altitudes, and with various oxygen-nitrogen mixtures. In resting subjects it was possible to predict the saturation with an error of 3 per cent within the range of conditions studied. Arterial oxygen tension and saturation fell in exercise as judged by samples obtained after exercise ended. The precise microanalytical methods now available call for a reassessment of the extent to which finger samples or ear-lobe samples differ in composition from blood drawn simultaneously from an artery. Such observations are particularly called for in exercise coupled with exposure to extremes of temperature.

Venous samples drawn during exercise may vary greatly in composition, depending on the duration and intensity of work, the physical fitness of the subject, the tissues drained by the vein punctured, and the external temperature.

There have been numerous studies showing the time-course of lactic acid in the blood during and after exercise and it is well recognized that the responses of blood gas concentrations and of pulmonary ventilation reflect changes in fixed acid. The first increments in lactate displace an equivalent amount of carbon dioxide and the arterial pH remains unchanged. Eventually, however, the lactate content effects a change in pH and other buffers, chiefly protein, supply base for neutralization, keeping pH changes relatively small. This question has had the attention of Turrell & Robinson (155) whose subjects reached a blood lactate of 22 m. eq. per liter, with an arterial pH of 6.97. Their calculations indicate the magnitude of the contribution made by hemoglobin and plasma protein to the base required for neutralizing lactic acid.

During 1936 and 1937 Edwards in this laboratory observed that the ratio of lactate between red cells and plasma was somewhat less than that of chloride in the resting state and was much less immediately after anaerobic work. Dependent dislocations of other anions including bicarbonate and chloride were suspected but Edwards' untimely death prevented the completion of this research. Recently Johnson and associates (156) have rounded out these experiments and have demonstrated that lactate diffuses slowly from plasma to cells, requiring about ten minutes to reach equilibrium at 37°.

The "standard" values for the blood of trained athletes are the

same as those of other healthy young men (157). Six months' athletic training did not affect oxygen and carbon dioxide combining capacities nor carbon dioxide tension. The trained men could do more work anaerobically, accumulate more lactic acid, and reduce alkaline reserve to a lower level.

Among pertinent reviews that should be mentioned is that of Comroe (158) dealing with the hyperpnea of exercise.

*Comparative physiology.*—Gas transport in the blood of species that vary widely in biological characteristics and in their preferred environments is largely an unexplored field in which Krogh has been a pioneer (159). With the improved techniques born of war-time research new opportunities are offered. Mammals and birds have similar problems of gas transport and the systems evolved for supplying oxygen to tissues are, as a first approximation, the same. Hemoglobin, however, varies greatly in its physical and chemical characteristics from one species to another and even in the same species from birth to maturity, demonstrated years ago by Barcroft and several associates including F. G. Hall (160). Hall has continued researches in this field, showing in particular how some animals native to high altitudes have blood with properties peculiarly adapted to successful living at diminished oxygen pressure (161). The contrast with low altitude birds was particularly striking. Those native to high altitude had blood with high affinity for oxygen while the blood of birds of low altitudes had a very low affinity for oxygen. Rostorfer & Rigdon (162) confirmed this and proved that duck's blood surrenders nearly all its oxygen at a tension of about 10 mm. Hg. They found that oxygen combining capacity of duck blood and its affinity for oxygen increase with age; in adults the oxygen combining capacity is 15 per cent higher than the color index indicates.

Respiration in diving mammals has been reviewed by Irving (163). Polycythemia characterizes some diving mammals (164). The mink, for example, was found to have an oxygen combining capacity of 26 volumes per cent while the oxygen dissociation curve was within the range of nondiving mammals: half-saturation corresponded to an oxygen tension of 37 mm. Hg. at a carbon dioxide tension of 40 mm. Hg. In other respects the blood was similar to that of other mammals. Such investigations point to the need for great caution in applying to man lessons learned from animal experimentation. One can imagine how confused we might be if our



knowledge of respiratory regulation and blood gas transport had been based on experiments on beavers and muskrats rather than on dogs and cats.

The transport of gases by fish exhibits phenomena unknown in higher animals. Irving and others associated with him have added much to our knowledge of how fish survive in waters varying greatly in temperature and in oxygen content. Variations in Bohr effect, in kind and amount of hemoglobin and in metabolic rate account for the ability of the trout to be active in cold mountain waters and its inability to survive in warm waters low in oxygen tension where the catfish thrives (165 to 170).

Reference is made to a number of papers dealing with fetal blood gases (171, 172, 173) that have come from Barcroft's laboratory since his review appeared (174).

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LABORATORY OF INDUSTRIAL PHYSIOLOGY  
GRADUATE SCHOOL OF BUSINESS ADMINISTRATION  
HARVARD UNIVERSITY  
BOSTON, MASSACHUSETTS

## WATER METABOLISM

BY EDWARD F. ADOLPH

*Department of Physiology, School of Medicine and Dentistry, The University  
of Rochester, Rochester, New York*

This review abstracts information about water metabolism as it appears in the published records of the past two years. The study of water metabolism has expanded greatly in the last twenty-five years if one counts the number of investigators who write on the subject. A corresponding expenditure of time and energy in investigations is evident. While reliable data are accumulating, it is not certain that new and unifying concepts and generalizations are keeping pace.

Why are physiologists interested in water metabolism? The following general objectives can be defined: (a) Studies have been made of discrete physical and physiological processes, illustrated by permeability, secretion, absorption, and evaporation. Elaboration of this interest appears as organ physiology. (b) Integrated and correlated activities have been studied whereby water balance is achieved. The role of water in the animal economy is thereby ascertained from quantitative studies of several processes and their interrelations. Much is gained by comparisons among species. (c) Disturbances found in disease, illustrated by alimentary obstruction, edema, diarrhea, and diabetes insipidus, have been studied. Functional tests are devised, therapies are tested, and disorders are reproduced experimentally in part. (d) Practical needs were exaggerated during wartime in particular directions, i.e., life in deserts, survival of castaways at sea, and work in hot industries. In rare instances all these interests are represented in a single investigation.

General and comprehensive reviews of contributions up to 1944 are provided by Peters (1) and by McQuarrie (2). Special subdivisions are also dealt with as follows: partitions of body fluids (3), exchanges in bowel (4) during disease in general (5), pregnancy (6), water balance (7, 8, 9), water excretion (10), and role of the neurohypophysis (11, 12, 13).

### TURNOVER OF WATER

Most living organisms preserve their water contents by restriction of evaporation and by replacement of water as fast as it is



lost. The rate of loss, partly at the mercy of environmental influences such as temperature, is exaggerated in the sweating man (14, 15). All air-breathing animals evaporate water, hence, some loss is unavoidable. Thus attention is called to the several processes which constitute routes of water expenditure, i.e., saturation of respired air, evaporation as a transfer of heat, and urine as a carrier of solutes.

A total aspect of water exchanges is represented by the measured water turnovers of various species under standard conditions [(9) p. 75]. Among thirteen terrestrial species from mouse to elephant, the fitted equation was: rate of turnover (ml. per hr.) =  $0.01 (\text{gm. of Weight})^{0.88}$  [(9) p. 270]. Among assorted aquatic species from ciliates to crabs, rate =  $0.016 \text{ Weight}^{0.78}$ . Evidently body surface area, which is in general proportional to  $\text{Weight}^{0.67}$ , is not the sole factor in water exchanges, but tends to be more important than body mass in small aquatic organisms.

*Intakes of water.*—In deserts, human intakes are greatly exaggerated as sweating becomes rapid; intakes up to twelve liters per day are reported (14). Average intakes depend largely upon air temperature, exposure to sun, and activity. In combat areas of North Africa a man ordinarily consumed two to four liters per day in warm weather (16).

Requirements of water by men on life rafts were carefully assessed (14, 17). Various sources of water for men surrounded by ocean were explored; in general, desalination by ion exchangers (18), distillation in solar stills (17), and catchment of rainfall (14) are available in addition to bottled water. Fish juice is not usually obtainable at sea, and whole fish do not add to man's net water supply (19). Sea water itself is an improper intake by any route, even when mixed with fresh water (19, 20, 21). Reasons for this are discussed under EXCRETION.

Voluntary dehydration prevails in all animals exposed to heat (14). Men, dogs, cats, rabbits, rats, mice, and guinea pigs with water readily available will sit in hot atmospheres that rob them of body water without drinking enough to replace what has been lost. In man voluntary dehydration is related to gastrointestinal discomfort (nausea, anorexia). The voluntary intake of man increases with acclimatization to heat (22), but usually covers only half the sweat loss. That the dehydration is physiological is shown by the improvement of physical performance after forcing enough water

to replace sweat losses (23). Another form of statement is that thirst, the urge to drink, is insufficient to keep the body in optimal state of hydration (14). In dogs and cats, drinking becomes adequate to maintain body weight after 2 to 4 per cent of the weight has been lost during panting. In rodents, almost no water is drunk throughout exposure to barely tolerable heat and the animals perish, partly from dehydration, while ignoring water.

Following desert dehydration, men given water drink it rapidly for the first few minutes; later they drink very little (14). If the dehydration does not exceed 2 per cent of the body weight, replacement is practically complete, as it is in the dog. If dehydration is greater, the replacement slowly brings the water content to that of the men similarly exposed but with water *ad libitum*; until meal time, when replacement to original body weight becomes complete.

A partial account of thirst has again been presented (14), but little progress has been made in localization of the sensations and urges that concern it. Dogs given pitressin reduce their water intakes below amounts that allow them to stay free of signs of thirst without the pitressin (24). When given water by stomach tube, the dogs that are slowly absorbing pitressin tannate refuse to drink any water for as long as forty-eight hours. Those organisms (frog, earthworm, ciliate) which do not take water by an alimentary tract nevertheless absorb it faster in dehydration than otherwise (9).

Further facts are reported about water intake. Kourilsky *et al.* (26, 27) finds in men with diabetes insipidus that restriction of water intake for two to four days is followed by voluntary intakes that bear no relation to the ones before restriction. Sometimes the polydipsia disappears, sometimes it is exaggerated. Rats rendered diabetic by pancreatectomy show marked increases of water intake during the first three weeks thereafter (28). If allowed to select their own diets, they subsequently stop eating carbohydrate and lose the polydipsia and polyuria. It is well known that water intake is reduced whenever food is eaten in less than usual quantities. This phenomenon is again illustrated by rats given qualitatively restricted diets of sucrose or glucose (29).

#### WATER EXCESSES

Water contents of the animal body greater than those ordinarily maintained may be produced by alimentary administration

(usually by stomach tube), by intraperitoneal injection, or by intravenous injection. Steady influx is obtained in man by swallowing 20 to 200 ml. of water every ten minutes (30, 31), a procedure that can be continued up to seven hours. After two hours the rate of output exceeds the rate of intake by 8 per cent. This phenomenon is attributed to the excretion of chloride and the attainment of a deficit of it. Output equals intake (isorrhea) when sodium chloride is present in the ingested fluid in certain concentrations (32). There are three such concentrations, 0.14, 0.6, and 1.7 per cent, in both man and dog. The excretion of water is accordingly believed to respond to both the excess of water present in the body and the load of solutes in the plasma. In a variety of circumstances, the rate of water excretion is proportional to the excess of water in the whole body (9, 33). Surgical patients who receive intravenous saline sometimes retain the ordinarily isorrheic solutions, and do not tolerate administration of much salt (58). When rabbits are given intravenous infusions isotonic with plasma, excretion of the water in urine appears to be intermittent (34, 35). The blood is most dilute just before each of three peak rates of urinary flow is attained. It is thought that the exchanges between plasma and tissues undergo hourly oscillations.

A syndrome involving water retention in man has been described by Kourilsky *et al.* (36). It is ascribed to malfunction of the hypophysis which excretes an excess of anti-diuretic hormone, thus preventing elimination of sufficient urine. Even 1,500 ml. of water elicits no diuretic response. The excess of water produces the picture of obesity instead of that of edema.

*Adrenals.*—The ordinary response to water administration is water diuresis that removes, during three hours, the water gained. The water diuresis fails almost completely in adrenalectomized rats (37, 38). If rats given water by stomach tube are killed, little more water is found in the stomach and intestine of adrenalectomized animals than in controls (38). Within the remainder of the body about as much of the administered water is retained as in nephrectomized rats. This follows both from weight increment and from analysis of the carcass for water. The volume of distribution of thiocyanate (believed to be extracellular volume) expands, but the inferred intracellular volume and plasma volume do not expand (38). The retention may be blamed upon both the accommodation of water outside the vascular space and an overactive reabsorption of water in the kidneys.

A diminution of water diuresis, in the first few weeks after extirpation of the adrenal medullae of rats, was reported by Stein & Wertheimer (39). Upon approximate repetition of the experiment in rats, Gaunt *et al.* (40) find no difference with and without the adrenal medulla but may not have tested the rats soon enough after operation. Hence, it is not settled that the medulla affects the response to excess water in the body, but when epinephrine is injected into normal rats, water diuresis is more prompt and greatly enhanced (40, 41). In adrenalectomized animals epinephrine enhances diuresis to a lesser degree, probably through its vascular effects in the kidneys.

Adrenalectomy, involving absence of adrenal cortices, greatly delays water diuresis in cats (42) and in rats (40, 41). Treatment with desoxycorticosterone removes the deficiency of excretion, but saline does not. The sterone may influence the distribution of water between plasma and other fluid compartments. Patients with Addison's disease likewise show almost complete absence of water diuresis (43).

*Water intoxication.*—Toxic signs appear when a certain excess of water is present in the body, usually relative to solutes, particularly sodium salts. Indications are decreased heart rate, convulsions, and cramp. For the several species in which it has been experimentally or accidentally produced it is known to depend upon the load or excess of water rather than upon the rate of administration or excretion of the water. Adrenalectomy or injection of pituitrin favor the onset of intoxication because excess water is not excreted readily. Desoxycorticosterone acetate or sodium chloride raise the tolerance for water excess (37).

It is found that resistance to the intoxication is acquired by rats to which water is repeatedly administered (37). Water was given by stomach tube in increasing doses on five consecutive days. The administration of the tube was only a small part of the situation to which adaptation occurred. Water diuresis also increases in velocity but does not relieve the body of enough water to account for the tolerance to its presence. When the adrenal glands are removed, the tolerance is acquired only in small part; it is postulated that the distribution of the excessive water in the body is an important item of protection.

The convulsions produced by water excess are accompanied by unusual brain waves (44). Even before convulsions materialize in intoxicated rats, slow waves of a frequency of one to three per

second appear at intervals and also convulsive spikes. The latter, which are signs of more extreme intoxication, as well as the patent convulsions can usually be abolished by administration of desoxycorticosterone.

#### DEHYDRATIONS

Water deficit is most rapidly produced in man by sweating evoked on exposure to high temperatures. Terrestrial animals that pant (dog, cat) can be similarly dehydrated, but in those that do not pant much (mouse, rat, guinea pig, rabbit, and insects), studies of dehydration depend on induced diuresis or acceleration of cutaneous evaporation (14).

The man or dog deprived of body water suffers "dehydration exhaustion," which is believed to represent a deficient circulation from decrease of circulating blood volume (14). The blood loses two to three times as much of its volume as does the body as a whole. Salivary flow meanwhile diminishes, so that the man who has lost 8 per cent of his body weight is spitless. Urine formation and sweat formation persist even during greater degrees of dehydration. The dehydrated individual also becomes intolerant to heat, since the circulatory system no longer carries heat to the surface for dissipation (23, 45, 46).

Even dehydration amounting to 2 to 4 per cent of the body weight has measurable consequences; the heart rate increases (47) especially in the standing man (14), the deep body temperature usually rises (23), and overbreathing may develop (14). Stroke volume of the circulation appears to diminish (48, 14). Exhaustion is manifested in the inability to perform prolonged work, in mental instability, and in mental depression (14, 49). Persistent esophageal movements, once thought to characterize the dehydrated individual, are not found in man (50). In dogs the blood shows marked concentration of all its elements (51, 62). Other signs of dehydration also appear in dogs (52, 62). In rats, moderate deprivation of water greatly increases the motor activity of running (53). This is regarded as a restless seeking of water. Thiouracil gives a small apparent protection against dehydration in rats allowed limited water intake (54). With this substance they diminish their body weights more slowly during two weeks, and develop larger thyroid glands. This fact suggests that metabolism and physical activity are reduced by the thiouracil. In contrast, dogs with diabetes insipidus tolerate water privation very poorly (55).

Dehydration in man cannot be repaired by any known means except by replacement of body water. If water be drunk, recovery from all signs of simple dehydration is prompt and dramatic. In disease, and especially among children, it is often impossible to give water by mouth; in such individuals infusion is resorted to, preferably by stomach (56) by vein (57 to 60), or by peritoneum. The degree of bodily hydration must then be estimated by observations upon the blood, the urinary excretion, the body weight, or other available criteria, none of which is easy to interpret.

It must be emphasized that dehydration is not merely a change in ratio of water to all other constituents, but that internal distortions result (61). In addition, other substances or stresses are often added while water is extracted, yet the physiological state is still termed dehydration.

Water deficits of amounts up to 11 per cent of the body weight have been studied in man (14). Other mammals survive quick dehydrations of 17 to 20 per cent (14) and slow dehydrations of 20 to 30 per cent of the weight [(9), p. 201].

For men in deserts, sea, and air, planned water supplies must be adequate if dehydration is to be avoided. Requirements of man can be estimated accurately in accordance with climate and physical activities (14, 63).

Is the castaway at sea preserving his water content more adequately when he eats or when he does not? An answer is not easy to obtain, because neither body weight nor tissue water content is a direct measure of body water content. The best that can be done is to compare the relative quantities of water and other substances lost during water privation and to ascertain how much water can be retained in a recovery period. Gamble (20) estimated that in starvation 535 ml. of water are required per day to balance the output. If 500 ml. of seawater be ingested, the urinary volume increases to an extent that no favorable balance remains. Diluted sea water has no metabolic advantage over the fresh water that diluted it, but if glucose or sucrose be eaten, the minimal urinary flow is diminished by virtue of the sparing action upon protein catabolism, so that less urea and sulfate require excretion in urine (19, 20). When fish is eaten, the water contained in it is barely sufficient to excrete the protein metabolites formed from the food.

Some of the same relations were illustrated in dogs (52). Amounts of protein of 1 gm. per kg. per day can be ingested during

water privation without change of nitrogen excretion, but 2 gm. or more are deleterious because proportionately larger urinary volumes result. Hence, protein food increases body dehydration and decreases the period of survival. But sucrose or glucose diminishes dehydration and prolongs survival without water. Ingestion of fish slows the acquirement of water deficit over that with dry protein, but not over that with no food, in spite of the higher concentrations of the nitrogenous catabolites in dog urine than in human urine.

#### WATER UPTAKE

In mammals the only natural route of water gain from outside the body is through the alimentary tract. If water is ingested into an empty tract, it is passed rapidly through the stomach. New analyses confirm older ones that in the dog, absorption of a dose of water is complete in forty minutes (64) and in the rat, in somewhat less time (38). After ten minutes about half of the administered water is still in the rat's stomach (65). Hypophysectomy delays water diuresis and also allows fluid to remain in the alimentary tract for at least one hour (66).

When distilled water (2 per cent of the body weight) is given to rats, anesthetized or not, the osmotic pressure of the ingesta quickly mounts (65). Even in the duodenum the fluid has nearly attained isotonicity and isochloricity at the end of ten minutes.

Movement of water through the intestinal wall has been intensively studied. When deuterium oxide is added to salt solutions in isolated ileac loops of anesthetized dogs, it is found that during periods of nine to eighteen minutes, a strong salt concentration retards the escape of deuterium oxide to a small extent (67). In contrast, the initial movement of water into the gut is nearly independent of salt concentration. The rates of movement of deuterium oxide are much greater than predicted from diffusion, and in some circumstances are the reverse of those predicted. Evidently water flows concurrently in both directions, and is controlled in two different patterns of forces.

The fact that water is absorbed from autogenous serum placed in the ileum of anesthetized dogs has again been confirmed (68). Although no concentration gradients initially exist between gut content and circulating blood, water moves into the blood. Moreover, it moves faster than some solutes and slower than others, so that the originally isotonic solution becomes slightly hypotonic.



Sodium and chloride appear to be absorbed disproportionately faster than the water of the serum. The tendency of the gut to modify an isotonic salt solution into a hypotonic one is abolished by adding mercuric chloride or sodium arsenite (69). The fact that faster water absorption occurs with greater hypotonicity indicates one property of the driving forces concerned in water transfer. Water sometimes enters the gut in the presence of hypotonic sodium salts. When the plasma is made hypertonic by putting 5 per cent sodium chloride solution into the dog's vein, the gut fluid increases in concentration but does not exceed the total concentration of the modified plasma (70). Meanwhile water may travel either into or out of the gut. On the whole it is safe to conclude that osmotic pressure is generally dwarfed in comparison to other forces in the exchange of water and inorganic ions across the intestinal wall.

#### NONEXCRETORY WATER LOSS

In air-breathing animals, the losses of water can be conveniently divided into loss by evaporation and loss as liquid. In mammals the latter occurs through sweating and through urine formation; the former from respiratory surfaces. Evaporative losses are often termed insensible, which is a practical but not a physiological subdivision; for often much sweat that is secreted as a liquid is evaporated before it can be measured.

*Evaporative loss.*—Ordinarily evaporative loss is measured under conditions of coolness and quiet such that no sweat is expelled. Man (71) or other animal (72, 73) is placed on a sensitive balance and weighed at frequent intervals.

The rates of total evaporation from rats, mice, and rabbits are markedly reduced by denying them water and food (72). Sleep also reduces the rates; denial of intake induces sleep, so that the reduction may be chiefly or entirely due to reduction of bodily activities. Strict partition of the losses between lungs and skin is obtained by leading the expired air through some device for catching the water vapor, such as a freezer. Usually over half of the evaporative loss is from the skin. It is not known that species differ in this proportion; the rat is similar to man (73).

It is agreed that the rate of evaporation from the respiratory tract is proportional to the ventilation rate (74, 75), but evidence is presented that the air expired may not be saturated (76, 77),

The difficulties are in avoiding condensation in valves or other apparatus and in determining the temperature at which the air leaves the respiratory passages (74). Warmth and humidity of the inspired air are factors, in that they modify the temperature of the outer respiratory passages. At simulated high altitude the saturation of the expired air differs by not more than 10 per cent from that prevailing at sea level in subjects breathing dry oxygen (75).

Insensible water loss in mammalian skin represents evaporation from layers of tissue below the skin surface. Renewed efforts have been made (78, 79) to trace the origin of the vapor, and particularly to understand the important retardation of the rate of evaporation as compared with that from a free water surface. The rate of evaporation from the skin at 33°C., about 6 mg. per cm.<sup>2</sup>, is the same for dead as for living skin, Negro and white, defatted and preserved. If the epidermis is removed, as by formation of a cantharides blister, the rate increases about eightfold and is similar to the rate of evaporation from exposed interior tissues. The epidermal layer itself, after its isolation, retains the ability to retard evaporation (78) for at least two months (81). Living skin, in which the sweat glands are atrophied (ectodermal dysplasia), allows the same rate of evaporation as normal skin. When warmed, evaporation from the living skin increases only to the same extent as from dead skin (79). Mild chronic trench foot produces no modification of the rates of evaporation (80). Isolated and killed skins retain some of the regional differences in rate of escape of moisture through it that they exhibited during life (81). These differences are not proportional to thickness of epidermis or its fractions. Various vegetable fruits having waxy coats show comparable low rates of evaporation (78). In hyperkeratosis the insensible loss is increased three- to tenfold (82). At first it might seem that thicker keratin would further retard evaporation, but keratinization itself involves loss of water from the drying skin and also makes for discontinuity of the granular layer (82). Hence, there is probably no contradiction to the generalization that the corneum is responsible for slowness of evaporation in the skin and that a layer of minimal thickness gives as much protection as a thick layer.

Comparisons among diverse organisms reveal the importance to the organisms of cuticular protection against evaporation (83).

In general, the majority of aquatic animals have no such protection, so that amphibia (84), annelids (85), and many others dehydrate at enormous rates when exposed to air not saturated with moisture. But mammals, birds, reptiles, many insects (83, 86, 87) and shelled mollusks (85) withstand great evaporating powers for surprisingly long times. In insects, the cuticular lipoids appear to be the crucial elements retarding evaporation (87). A means of opposing evaporation may be the most important element for survival in terrestrial situations (83). In frogs, the differences of temperature between body and air are proportional to the rates at which evaporation from the body is occurring (84). In insects, it is found that within limits the rates of evaporation of water are proportional to the saturation deficits of the air (83). In this way, the effects of humidity are separable from those of temperature and duration of exposure, and it is possible to show the effectiveness of evaporation in lethal dehydration of diverse species of arthropods, including those that carry infections to man.

*Sweat production.*—Local sweating is studied by allowing the sweat to evaporate and collecting the distillate, or by observing its presence at pores. With ferric chloride (88) or starch and iodine (89, 90) on the skin of man or rat foot, prints may be obtained of the individual pores that give forth liquid. The droplets issuing may also be collected and analyzed (91). So far no independent evidence has been used to check the accuracy of these methods and their results. Regional distributions of sweating in man are compared by absorption and weighing of sweat under capsules applied to the skin (92).

The rate of general sweat production is measured by loss of weight, the individual being dry at each weighing. Use of body water for the formation of sweat can for an active man in the desert amount to as much as 11 liters per day (14). In hot moist atmospheres, over 3 liters of sweat may be secreted per hour; in this situation the sweat runs off and produces relatively little heat loss (22).

It has been demonstrated in well-controlled experiments, both outdoors (14) and indoors (22), that the rate of sweating is not affected by water intake nor state of bodily hydration, both at rest and at work. There is no significant saving of water by denying it to the man, since any water deficit is repaid later. This conclusion relates to sweating only, and does not apply to evaporation in

cool surroundings. In moist heat (air at 92°F. and nearly saturated), men walking at a given pace sweated more after several days of acclimatization to the heat than they did upon first exposure (22, 93). Hence sweating becomes more readily aroused and plays a part in improving man's tolerance to heat. When first exposed to a hot dry atmosphere, a resting man begins to sweat before he can be accurately weighed (94). At five to fifteen minutes later a second acceleration of sweat production is found, maximal rate being attained at about twenty minutes. During six hours of work in any hot atmosphere, the maximal rate of sweating occurs in the second hour, after which the rate declines (95). Within six hours this decline often renders insufficient the effective cooling of the man. The decline is less in a dry atmosphere where the sweat is being evaporated than in moist air where the sweat is wasted. Sweat is secreted by working men even in cold surroundings (96), and in the interests of conservation of body water, it becomes desirable to shed clothing before the subject feels comfortably warm (97). The sweat is evaporated at the skin surface and condensed in the clothing, so that the water loss from the body is no longer proportional to heat loss by vaporization.

Sweat glands may be functionally deficient in men, numerous cases having been recorded as congenital absence of the glands (98). New evidence suggests that the glands may also atrophy in the individual (99). Such an atrophy might account for some of the occasional failures to tolerate heat after prolonged exposure to hot climates (45, 46). It is reported that during severe episodes of heart failure, the ability to sweat is almost abolished (100).

The composition of sweat is known to be very variable, and only a few factors that influence it have been distinguished. One factor is the salt intake, and concentrations of chloride in sweat of only 5 m.eq. per liter have been reported (101). It is found that the composition of sweat over a twenty-four hour period can be ascertained from the equation:

$$[Cl] \text{ of sweat} = \frac{Cl \text{ intake} - Cl \text{ of urine}}{\text{Volume of sweat}}.$$

The concentration of chloride thus found is equal to that present in the sweat collected inside a rubber glove. A similar computation is valid for total nitrogen of sweat.

Thus it is seen that water is lost from the general body surface

and from the respiratory surfaces. From both, evaporation occurs before the liquid has appeared as such. It is probable that the air in contact with the liquid becomes saturated at the temperature of the liquid. Over general body surfaces, evaporation is much retarded by the presence of protective coatings.

#### EXCRETION OF WATER

Rats receiving high-fat diets use less water to form urine than when receiving a carbohydrate-rich or a mixed diet (102). When slightly dehydrated and then given water by stomach tube, the fat-fed rats excrete urine more slowly than rats fed mixed diets. Phenol red excretion is not different, and nonrenal weight loss is unchanged. It is suggested that the change in renal output may be produced by impairment of liver function (fatty liver). Rats kept in air of constant temperature excrete less urine upon transfer from humid to dry atmosphere (122). The reverse also occurs, but not if the animals are thyroidectomized, which suggests that activation of thyroid gland may be responsible for the modification of excretion.

The transient anuria which often follows crush injuries in man has been reproduced, after muscular trauma, in anesthetized dogs, where it appears one and one-half hours after the injury (103). The same reaction is produced in rabbits by injecting human myohemoglobin at release of leg compression (104). In the rabbit, which has almost no myohemoglobin, crush alone does not produce anuria, nor does the pigment without crush produce it. Temporary renal insufficiencies in both dog and man are successfully treated by repeated peritoneal lavage (105). Following burns (106) and other types of injury, temporary suppression of urine formation is frequent. Urinary flow is diminished in dogs during deep anesthesia by either cyclopropane or ether (107). In this oliguria the filtration rate (estimated from creatinine clearance) and the plasma flow (estimated from aminohippurate clearance) are diminished in proportion to the urinary flow. It is suggested that the renal blood flow is reduced by vasomotor constriction of afferent arterioles.

Output of urine is moderately increased in hypoxia of men exposed to simulated high altitudes of 18,000 feet (108), but not of 10,000 feet (109). In each case outputs of inorganic substances are parallel, since sodium, potassium, and chloride increase with the

diuresis (108) and phosphate output decreases slightly when no diuresis appears (109). In anesthetized dogs subjected to hypoxia, oliguria is found (110), in confirmation of older results. If the kidneys are denervated the effect that comes on at 14,000 feet is abolished; yet at 24,000 feet oliguria then occurs, unless the adrenal glands are inactive. It is known that diuresis may commonly occur in dogs, especially when not anesthetized, during hypoxia. Excessive feeding of vitamin A increases the urinary flow of rats (111). Clearances of inulin and of diodone increase in proportion to the urine; this suggests a circulatory basis for the change of urinary flow. Free fluid then often accumulates in the abdominal cavity.

*Diuresis.*—Excessive rates of urine formation are experimentally aroused by certain pharmacological agents, by high concentrations of excreted solutes, and by excess body water.

A comparative study of melamine, adenine, and formoguanamine showed this order of increasing diuretic potency in both dog and rat (112). The potency is measured as the reciprocal of the dose needed to elicit equal amounts of extra urine. These diureses are not inhibited by injection of pituitrin. Measurements of inulin clearances suggest that, in diuresis due to xanthines, faster filtration occurs in renal glomeruli and water reabsorption is at the same time somewhat diminished (113). Chloride excretion is augmented along with water excretion by salyrgan, as well as by caffeine and numerous other diuretics (114, 113).

In the excretion of excessive amounts of solutes, diuresis consists in forming considerable volumes of highly concentrated urine. In rats, maximal urinary concentrations are obtained by feeding a protein diet without water for several days (115). Under such conditions urea reaches a concentration of 14 per cent in the urine; for young rats of 70 gm., even 16 per cent. When rats are poisoned with uranium nitrate the maximal concentrations attained, when water intake is limited, diminish from day to day. This type of concentration test therefore measures the known renal deficiency produced by uranium. Normal rats allowed water ad libitum produce urine about half as concentrated as the maximal. The same quantities of water appear in urine, whether the rats eat 20 per cent or 95 per cent protein; hence the urinary water output is not all obligated by the urea excretion.

Sulfate capable of raising urinary flow in rabbits 150-fold, is still nearly the most potent of solute diuretics (116). Renal blood flow and creatinine clearance increase during the diuresis.

In man the recognition of maximal urinary concentrations is applicable in the estimation of renal water requirements (20, 117). During dehydration the output of water is regulated almost solely by the solutes that are being excreted. This fact is also expressed in the relation known as minimal clearance, which states that rate of solute excretion is proportional to rate of water excretion. The relations among the diverse solutes are such that the highest concentrations of urea, which may reach nearly 6 per cent in man, appear only when salt excretion is small (118). Solute diuresis is readily produced during dehydration or hydropenia, and there is no apparent limit to the rate of believed reabsorption of water during the formation of urine; the concentration of the urine is limited but not its amount (119, 120). However, the total concentration falls slightly with increases in rates of urinary excretion. It is argued, though the evidence is far from complete, that maximal concentrations attained by the solutes present in renal passages do not directly account for the diuresis.

Much attention is being given to the manner in which excretion is regulated during the diuresis of water excess and to the intermediaries that are active. As to filtration and reabsorption, there is no one answer, for rabbits show increased inulin and diodone clearances during water diuresis while rats do not (121). In man it is found that inulin clearance is slightly increased during water diuresis, but diminished reabsorption is also a factor (30, 113). The marked changes of composition that occur in kidneys (rat) during diuresis may ultimately help to understand the processes of diuresis (159). The rate of water excretion augments reciprocally the clearances of many substances, as has again been illustrated in man (123). For nine substances the augmentations of clearance were very similar in man and were nearly maximal at urinary flows of 1 ml. per minute. The similarity among these selected substances points to a common factor in the excretion of all of them.

The conditions under which water diuresis can be modified are being elucidated. Diuresis is not yet found in fetal rats injected subcutaneously with water, though a concentrated solution of urea induces significant diuresis (124). Even normal fetal rats are demonstrated to be forming significant quantities of urine (125). In man the erect posture diminishes water diuresis compared with the diuresis while lying supine (30). Inulin, urea, and diodrast clearances are all moderately reduced when men are in an erect position.



Intravenous injection of adenosine triphosphate (0.25 mg. per kg.) diminishes water diuresis and induces other shock-like signs (126). Brief intense exercise, such as a sprint run, inhibits the diuresis; the inhibition is more reproducible in mornings than in afternoons, but does not depend on breakfast (127). Clearances of both inulin and diodone usually fall temporarily with the exercise but do not correlate quantitatively with the rates of urinary excretion (128).

During water diuresis in man the output of chloride often diminishes (114, 129), while hydrogen ion concentration and ammonium output increase (114, 129). Alcohol in so far as it acts as a diuretic, does so in the same manner as ingested water, decreasing the urinary chloride output (130).

Hot atmospheres, with light work, also inhibit water diuresis in man; water is readily diverted into sweat formation (14). It is found that even though water is drunk as replacement for the sweat lost, diuresis is still inhibited (131). It is not yet known whether the kidneys receive blood of the same dilution during work in both hot and cool atmospheres.

Water diuresis in dogs is temporarily inhibited by vomiting produced by any one of nine different emetics (132). The irritant materials are believed to induce a reflex change in renal blood flow. Barbiturate anesthetics likewise diminish the rate of formation of diuretic urine (133). When syncope follows prolonged maintenance of erect posture in man, water diuresis is markedly inhibited (134). After blood was transfused from an erect individual to a diuretic supine individual, his diuresis was also inhibited. Even a person with diabetes insipidus shows some inhibition; nevertheless, it is postulated that the neurohypophysis is concerned in the response.

Hypophysectomy greatly delays water diuresis in rats (66), making excretion of the water load as slow as it is after isotonic solution of sodium chloride is ingested. Administration of corticosterone restores the water diuresis, as it does after decrease of diuresis in riboflavin deficiency (191).

#### NEUROHYPOPHYSIS AND WATER DIURESIS

Great interest has centered in the neurohypophysis as a possible regulator of water excretion. It was early established that extracts of the neurohypophysis are ordinarily antidiuretic. It is

remarkable that the same action is obtained from extracts of neurohypophyses of all vertebrates that have been tested (11). Even some crustacea yield extracts of similar potency. It is, paradoxically, unlikely that the same renal processes are affected in all species; in reptiles and amphibia the glomerular circulation is impeded before reabsorptive processes are enhanced. Water exchanges are additionally affected in amphibia, for the same dosages of pituitrin also speed up water intake through the skin, doubly producing an excess of body water.

The antidiuretic action in mammals becomes diuretic for certain dosages of posterior pituitary extract; this reversal in rats is related to the rate of chloride excretion and to the chloride content of the body (135). Posterior pituitary extract itself promotes the rapid excretion of chloride, as do other diuretics, particularly xanthines.

A test for antidiuretic response in man consists in giving water by mouth and antidiuretic hormone by muscle (136). If more than 80 ml. of urine are collected in the period from one-half to one and one-half hours following, abnormal kidneys are suspected.

The action of antidiuretic hormone is believed to be due to enhanced reabsorption of water in renal tubules. Confirming evidence was found in the clearances of inulin and diodone in rats, which clearances were not significantly modified during pitressin antidiuresis (121, 137). They were, however, reduced by pitocin or by whole posterior pituitary extract in particular doses.

Dogs with hypophysial stalk section were studied to show that most of the water intake occurs in the hours following feeding, as it does in normal dogs (55). Food privation diminished the water exchange, but even eight days of inanition did not eliminate polydipsia and low urinary specific gravities. Water deprivation was a severe ordeal; on the second day food was refused, urine was still of low specific gravity, and blood serum was of markedly higher osmotic pressure. One dog died in this brief period of water privation, leading to the view that the high water intake in diabetes insipidus is unavoidable (55). Proof was adduced that several functions of the anterior lobe of the hypophysis were normal in these dogs, but the thyroid glands were extremely small, and possibly were engaged in compensating for shortcomings of the posterior lobe (138).

Antidiuretic activity in human blood has been surveyed in 300

women by the rat method (139). In about 20 per cent of nonpregnant women the blood showed such activity; it was absent during most of pregnancy but was found in 41 per cent of pregnant women near term and during the first week post-partum. Its occurrence was not related to eclamptic states.

A sensitive method of assaying antidiuretic material was reported by Hare *et al.* (140). Tissue extracts, blood, or urine were injected into a vein of a dog with experimental diabetes insipidus and compared with standard doses of post-pituitary substance. The effects upon ratio of creatinine concentrations in urine and in plasma (U/P) were ascertained. It may be safely assumed that P does not change over hourly periods of time, wherefore increment in U may be compared when known and when unknown are administered. By this method it was shown (141) that the supraoptic region of the dog's hypothalamus contained as much as 20 per cent of the concentration of antidiuretic activity that the post-pituitary contained. When the supraoptic nuclei were caused to degenerate, by hypophysectomy or by cutting the stalk of the pituitary, the hypothalamus lost much of its antidiuretic activity.

Antidiuretic substance is released from the dog's neurohypophysis by injection of solutions that raise the osmotic pressure of the plasma, as detected by changes of creatinine clearance (142). After 5 per cent solution of sodium chloride is injected into a rat, 40 per cent of the antidiuretic activity may be lost in twelve hours (143). When rats are allowed to drink only 1.5 to 2.5 per cent solution of sodium chloride for several weeks, all the assayable antidiuretic activity may be lost from the neurohypophysis. At the same time mitosis of pituicytes is found to be most active (144). In cats the antidiuretic potency of the neurohypophysis gradually decreases after section of the supraoptic tract (145).

Much evidence has been accumulated to indicate that the release of antidiuretic hormone is ordinarily mediated by central nervous impulses (12). The pathways to the neurohypophysis, activated from unknown receptors, are in the supraoptic tracts. Acetylcholine is necessary for the transmission of nervous impulses to the hypophysis and is itself an influential agent. Again, it is confirmed in dogs that only section of the supraoptic tract confers permanent polyuria (146).

A successful distinction has been made between the sudden temporary inhibition of water diuresis and the more lasting inhi-

bition, both of which are obtained reflexly (147). The sudden inhibition that appears after faradic stimulation on the dog's flank is abolished by denervating adrenal glands and kidneys. The slow inhibition is abolished after section of the supraoptic tract (148), or by injection of epinephrine; which procedures are believed to prevent the release of antidiuretic substance from the neurohypophysis.

Reference was made to the fact that acetylcholine inhibits water diuresis only when the neurohypophysis functions. That morphine acts similarly was demonstrated by administering to a dog two doses of water, three hours apart (64). The dose of morphine was either 2.5 mg. per kg. by vein or 5 mg. per kg. subcutaneously. Ninety per cent of the water administered by stomach (40 ml. per kg.) was found in the urine during three hours, but after morphine only 12 per cent was returned. At the same time chloride excretion was markedly augmented. Of water given by vein (25 ml. per kg.), 110 per cent appeared in urine without morphine, and only 14 per cent with it. Hence the effect of the drug is upon excretion and not upon absorption of the water, which is complete within forty minutes before the drug is injected. The extirpation of adrenal medullae had no effect upon the diuresis nor upon the influence of morphine. Entire hypophysectomy reduces the return of administered water to 50 per cent, and likewise reduces the return after morphine to 8 per cent. High section of the pituitary stalk in ten dogs completely precluded any effects of morphine upon diuresis, whether the water be given by stomach or by vein. In these dogs, morphine did not modify the effect of a minimal dose of post-pituitary substance, hence did not potentiate the latter. Since potentiation did not occur, morphine is concluded to have its antidiuretic effect by releasing substance from the neurohypophysis. Parts of the same evidence have been gathered for yohimbine (dog) (149) and nicotine (rat, man) (150); in both, the antidiuretic effect disappears when the neurohypophysis ceases to function.

A differential test for diabetes insipidus of hypophysial origin has been applied to patients (151). When a hypertonic solution of sodium chloride is infused by vein during forty-five minutes, polyuria is not inhibited if the hypophysis is injured. This test is validated in hypophysectomized dogs and in patients. Another more sensitive test, which is also more difficult to carry out, is as follows: estimate filtration rate by inulin clearance, measure chlo-

ride concentrations in plasma and in urine, and compute chloride filtered and hence chloride reabsorbed, in successive fifteen minute periods. In normal individuals or in insipidus patients given post-pituitary extract the ratio of reabsorbed chloride to filtered chloride was unchanged during forty-five minutes of infusion with 2.5 per cent solution of sodium chloride. Simultaneously the ratio of urinary chloride to plasma chloride did change. In each case the patients with believed hypophysial damage showed the reverse effect.

A review of clinical diabetes insipidus is given by Jones (152). Some forty-two patients diagnosed as having diabetes insipidus of diverse etiologies were compared. Any one of them failing to respond to a concentration test by producing urine of high specific gravity was considered to have presumptive supraoptico-hypophysial damage. The clinical states of many of the patients were assisted by low-salt diets, some by thyroidectomy. Kourilsky *et al.* (153) present data to show that polyuria may prevail for some time, in certain patients, without corresponding polydipsia.

#### WATER CONTENTS AND REDISTRIBUTIONS

*In whole body.*—Analyses of water contents of whole organisms are accumulating [(9), p. 196]. So far no marked differences have been observed within mammals. Among diverse phyla and classes of animals diversities are known; partly they are related to the fat contents and to the various proportions of tissue types that are represented. In guinea pigs an adequate series of analyses shows that water content is a constant proportion of the fat-free body weight over a wide range of adult body sizes (154).

In rabbits the volume of distribution of deuterium oxide was ascertained after its injection (155). Although the deuterium exchanges with hydrogen in other compounds than water (155, 156), about 95 per cent of the exchangeable hydrogen is in the water. Hence, the volume of distribution is believed to be the total water present in the body; in human erythrocytes it equals the total water contained in them (156). In nine rabbits the total bodywater, as analysed by drying the residue, agreed with that found after deuterium oxide injection with a mean difference of 2.8 per cent (155).

*In individual tissues.*—In general it can be observed that

experimentally-induced changes of water content often represent replacement of tissue materials by something else, such as fat. Some effects that have been tested upon tissue water contents are: high fat diet (157), corticosterone (158), dehydration and hydration (159), pyloric obstruction (160), choline (161), cancer (162), concussion (163), and season (164). In each study particular kinds of tissue were analyzed, often in relation to fat-free weight. In addition, recent analyses are available of human skin (165), dog tendon (166), and several tissues of guinea pig (154).

Estrogen is specific in modifying the water content of uterus but not of liver and skeletal muscle in rats (167). Castrated females were given estradiol in oil, which exerts continuous action for several weeks. Both endometrium and muscle share in an edema which is maximal at six hours after treatment begins; thereafter cell division and oxygen consumption accelerate while the water content diminishes half-way to normal, at which level it remains for at least four days. In another series of rats in estrus, the uterine water content was found to be maximal at seventeen hours and fully recovered in two days (168). Progesterone injections produce only a small increase in uterine water.

Compartments of the animal body have their volumes estimated by adding substances of limited distribution. Recent measurements include mannitol in man (169); inulin, sucrose, and thiocyanate in nephrectomized rabbit and dog (170); thiocyanate and T1824 in man (171, 172), in dog (173), and in rabbit (170, 174); and T1824 in man (175) and dog (176, 177). Mannitol, inulin, and thiocyanate do not have equal volumes of distribution, as was formerly supposed. It is believed (170) that the extracellular volume is best measured by sucrose. In intestinal obstruction both plasma and extracellular volumes are found to diminish, the fluid passing from them into the intestine (174). In leg trauma the loss from plasma alone appears to be nearly sufficient to account for the increase of leg volume (177, 178). In hemorrhage the fluid that flows into the plasma is shown to come from "extracellular" spaces (173), and its concentration of protein is considerably below that already in plasma. Even hypoproteinemic dogs replace part of the plasma rapidly. If blood from a hemorrhage be replaced by reinjection, a second hemorrhage of equal amount leads to a slower replacement from extravascular sources (176). Extracellular and

intracellular volumes are frequently computed from tissue analyses of potassium and chloride (61, 179), and numerous tentative conclusions are drawn from those computations.

*Transudation.*—Exchanges of fluid between blood and extravascular spaces are tentatively believed to be governed by the forces of hydrostatic pressure and of colloid osmotic pressure. This is the Starling hypothesis, which remains tantalizingly probable but unproven. Some possible additions to the Starling hypothesis are reviewed (189). Several sets of measurements show that each gram of albumin added to plasma retains 18 ml. of additional plasma volume (190). In renewed study of single blood vessels of frogs, a pipette is placed into a capillary and another outside the capillary (180). An electrical potential difference imposed between them (inside positive) brings fluid into the capillary in a mechanically injured area. Cessation of flow of electrical current allows filtration outward; flow outward is further augmented by making the outer electrode positive.

Filtration rates under venous congestion were measured in the human arm by means of venous blood samples, both at sea level and at simulated high altitude (181). No significant difference appears in the rate of fluid loss. After venous congestion enough edema fluid can be drained for analysis (182). With stasis pressure of 30 mm. Hg., the fluid contains 0.8 per cent of protein. In edema fluid, drained without added stasis from patients with cardiac failure, only 0.3 per cent protein is present.

Lymph flowing from a local area often indicates the formation rates and compositions of transudates. From the lungs of anesthetized dogs the flow increases with intermittent positive pressure ventilation (183). It ceases entirely if ventilation is effected without breathing movements. Injuries that bring on pulmonary edema are detected earliest in augmented lymph flow from the lungs. In legs of anesthetized dogs, rabbits, and goats subjected to thermal burns, the lymph issues much more rapidly and with increased protein concentrations (184). Blood flow, plasma loss, and lymph flow are all dramatically reduced by cold.

Volumes of the legs of anesthetized mammals that are subjected to burns (184), venous tourniquets (185), or mechanical trauma (177, 178) increase enormously, as is well known.

A study parallel to that of transudation concerns the exchange of fluid by isolated tissues. It is again shown that fatigued muscle



of frog swells faster in distilled water or in Ringer's solution than does rested muscle (186). Muscles of the whole frog leg lose water if exposed to an atmosphere of carbon dioxide (187). In isotonic solution of potassium chloride, the muscles swell (188); but if the frogs are previously adrenalectomized or if the muscles are poisoned with iodoacetate, the swelling is significantly less.

The transfer of fluid from one tissue to another is in need of systematic study from all the possible angles, of which the above are only a few. The forces that guide transfer may be more numerous than present theories of osmotic and hydrostatic pressures suggest.

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DEPARTMENT OF PHYSIOLOGY  
SCHOOL OF MEDICINE AND DENTISTRY  
THE UNIVERSITY OF ROCHESTER  
ROCHESTER, NEW YORK

## PHYSIOLOGICAL EFFECTS OF HEAT AND COLD\*

BY A. P. GAGGE

*Aero Medical Laboratory, Wright Field, Dayton, Ohio*

AND

L. P. HERRINGTON

*John B. Pierce Foundation, and Yale University School of Medicine,  
New Haven, Connecticut*

### FATAL EXPOSURE TO COLD

On July 10, 1945, a report<sup>1</sup> was submitted to Allied authorities which made notorious history. In this extraordinary report on the Dachau concentration camp (1), those who have an interest in the social responsibilities of science may gain new insight into the type of rationalization of those who support such activities. The subject of investigation in these discreditable experiments was the physiology of cold exposure. A full review of the credible portions of the work is warranted by reason of their intrinsic interest and the relative inaccessibility of the report.

The Dachau experiments were conducted by a certain S. Rascher, apparently a pharmacologist, under direct orders from Himmler, and with the collaboration of the medical division of the Luftwaffe. Their object was to determine the most effective method of resuscitation for sea or land exposure cases recovered with reduced body temperature and evidences of exposure shock. One section of the general plan provided for the exposure of nude prisoners to air temperatures of  $-6^{\circ}\text{C}$ . for as long as fourteen hours, a period sufficient to freeze peripheral tissue and to reduce rectal temperatures to  $25^{\circ}\text{C}$ . A more fully reported series provided for the experimental immersion in water at  $2^{\circ}$  to  $12^{\circ}\text{C}$ . of subjects either nude or dressed in standard flight equipment.

In these procedures not less than 103 persons were used, few of whom appeared to have survived the complete experimental program. In one table, seven deaths are specifically recorded. Because of the bearing of this record on absolute tolerance to extreme cold,

\* This review covers the period from July, 1945 to July, 1946.

<sup>1</sup> The Treatment of Shock from Prolonged Exposure to Cold Especially in Water. A report by Major Leo Alexander based upon an investigation of physiological experiments with human beings in German concentration camps. (1).



the average thermal data on these seven who died from the effects of tank immersion are given in Table I. From other data it may be estimated that during the course of an hour death resulted after a reduction of 13° to 14°C in average body temperature with a net heat loss of 700 to 800 kcal. This fatal degree of chilling is approximately eleven times the normal basal heat production per hour. (In comparison, deaths from heat exposure normally result from

TABLE I  
THERMAL DATA ON CLOTHED HUMAN SUBJECTS DYING AFTER  
IMMERSION IN COLD WATER

	Water Temp. °C	Time of Immer- sion	Time at Death	Rectal Temp. on Removal from Bath, °C	Rectal Temp. at Death °C
Average Range	4.6° (4.2-6°)	67 min. (53-98)	73 min. (53-100)	28° (26.7-29.2°)	26.8° (25.0-29.2°)

TABLE II  
COOLING OF CLOTHED HUMAN SUBJECTS IMMERSED  
IN WATER AT 4.5°C

Time, minutes	0	20	30	40	50	60	70
T <sub>rectal</sub> : °C	37.0	36.2	35.3	33.3	32.4	30.2	29.5
T <sub>skin</sub> : °C	35.0	22.0	17.7	17.4	16.8	16.4	16.2

an elevation of 6° to 7° in average body temperature with a net heat increment of 300 to 400 kcal.)

The cooling characteristics of the human body under extreme cold stress are not generally known for extreme exposure, hence we have reproduced a section of data in Table II, giving parallel rectal and skin temperatures for seventy minutes immersion at 4.5°C. water temperature for a subject clothed in flying equipment.

*Clinical observations.*—In both anesthetized and normal subjects, immersion in water at 2° to 12°C. was associated with a rousing effect marked by defensive movements. These effects were attenuated in anesthetized subjects but without complete return

of consciousness. Following the preliminary phase of stimulation of fifteen minutes muscular rigidity appeared, and increased with time. This rigidity effect disturbed the pattern of respiratory muscular action, resulting in labored breathing, incidentally characterized by marked distention of the nares. Rigidity was extreme in the arms, these members becoming fixed in a contracted position, tightly pressed to the body. After the body temperature was lowered still further, all rigidity suddenly ceased. Such subjects always died and resuscitation measures were unsuccessful. The validity of this sign (sudden relaxation) as an indication of the subject's arrival at an irreversible degree of cooling is stressed over any average value for total tissue cooling in terms of rectal temperature, or the terminal cardiac sign of total irregularity, including several cases of a presumed cessation of beat.

Although it is not rigorously demonstrated, these data strongly suggest that the proximate cause of death, heart failure, may ensue with or without prior decay of an actively organized central process of temperature regulation, strongly though ineffectually evidenced in the rigidity pattern. Under these circumstances, the survival data indicate that prior failure of the centrally organized response may be a critical and irreversible phenomenon in a sense distinct from cardiac failure including the terminal irregularities of action and apparent clinical death with cessation of beat. It is significant that no case exhibiting terminal relaxation recovered, despite a temporary persistence of heart action. Recovery after apparent cessation of beat during the period of rigidity was observed.

*Respiration.*—Dyspnea was a marked characteristic of the phase of muscular rigidity, the expiratory phase being noticeably prolonged and difficult. In later stages rattling and stertorous respiration appeared, frequently accompanied by oral vesicular foam. Auscultation, however, gave no definite evidence of pulmonary edema. Initial immersion greatly accelerated respiratory frequency but rates of 24 per minute were typical after twenty minutes of exposure. Oxygen saturation was not measured. At autopsy, however, venous blood was described as black, the blood in the right heart as dark red, and that in the left heart as bright red. Carbon dioxide concentrations in arterial and venous blood were greatly decreased. Especially interesting is the fact that in only two instances was respiration reported as failing simultaneously with cessation of heart action. In all other fatal cases, respiration

of a slowed but normal type, or in agonal gasps, persisted for periods up to twenty minutes after cessation of heart action.

*Heart action and circulation.*—On immersion, heart rates were accelerated from prior values of 80 to 90 per minute to 120 to 140. Since this acceleration also occurred in anesthetized subjects, it is not attributable to psychic factors. Peak rates were quickly reached, after five to fifteen minutes rapid reduction to normal levels occurred which were maintained until rectal temperatures near 34°C. were reached. Near this temperature a bradycardia gradually developed with a rate near 50 but regular and normal in character. On further cooling to an average rectal temperature of 30.5° (the range in ten subjects was 29.0° to 32.4°), a phase of total irregularity appeared suddenly, associated with atrial fibrillation in all cases with electrocardiographic records. This represented the terminal phase unless resuscitative measures were taken. On re-warming, a fast irregularity appeared which persisted until rectal temperatures (29.1° to 36.1°) were reached which were significantly above the critical point for irregularity during cooling.

Hemoglobin increases of 10 to 20 per cent were observed. The most striking changes found, however, were leucocyte counts of 26,000 per cu.mm. after sixty minutes of exposure, a persistent elevation of blood sugar by 80 to 100 per cent, and greatly increased values for blood viscosity. Heart failure is reported as the cause of death in all cases. The failure is attributed to direct cold injury and overloading due to blood viscosity increase. Values of seven to eight times the viscosity of water were reported, and this range may be contrasted with a normal value of 4.5 to 5.5.

*Resuscitation technique.*—Rapid rewarming of subjects by direct immersion in hot baths up to 50°C. (122°F) excelled all other forms of postexposure treatment by a wide margin. It has generally been supposed that rapid rewarming treatment is productive of shock. In theory, long exposure to cold should cause peripheral constriction, overloaded central circulation and consequently reduced blood volume. This, together with a deterioration of the temperature regulating mechanism caused by the hypothermia, might reduce the efficiency of adaptation to the increase in capacity of the peripheral bed produced by direct heating. Although this reaction must occur in some degree, it is quite clear that it is not productive of further shock. Clinical observations support the objective evidence of a rapid return toward a normal temperature and

circulatory status. When the unconscious subject (with rectal temperature of 25° to 30°C.) was first immersed in the bath (at 40° to 50°C.) a sharp outcry occurred immediately, despite the previously comatose state. Shortly thereafter the labored respiration and the generalized rigidity of the muscular system disappeared. Full consciousness returned and usually at a rectal temperature lower than the 31° at which it was usually lost during the cooling process. The warm immersion was usually for a period of ten minutes, or until spasm in the peripheral vessels was relaxed. This was followed by brisk rubbing and friction of the skin, after which the subject was wrapped in warm blankets. Under this routine the rectal temperature of a subject might be reduced to 25°C. (at a water temperature of 5°C.) in approximately one hour, and regain normal temperature in a further two hours as a result of ten minutes immersion at 40° to 50°C. and subsequent packing in blankets.

Among rejected treatments were the light cradle, heated sleeping bags, and friction. It was found these were not efficient in preventing postexposure drop of 2° to 4°C. in rectal temperature which resulted from the temperature equilibration of the colder periphery and the central circulation during slow rewarming, a phenomenon often associated with fatal collapse. The uneven peripheral heating of these methods was also considered as unfavorable. In like manner direct diathermy of the heart, as well as intracutaneous and intravenous strophanthine, lobeline, coramine and metrazol were found to be ineffective in the re-establishment of circulation.

Certain additional items of general physiological interest are reported. The first of these is the absence of glycosuria in the 500 cc or more of urine taken from the subject after the experiment or at autopsy, despite blood sugar levels elevated by 80 to 100 per cent over normal during a substantial part of the total cooling-reheating period. This suggests that there is either a rapid diuresis in very short initial and final portions of the exposure and recovery period with interim suspension of kidney function during the period of high blood sugar, or if the urine volume is a continuous accumulation, the sugar threshold must undergo considerable alteration during the period of body temperature change from 37° to 25° and back to 37°C.

In another respect the extraordinary quantitative effect on general cooling of increasing the immersed surface by less than 5

per cent of the total surface through submersion of the dorsal neck and head surface deserves comment. A rectal temperature of 26°C. was reached in seventy minutes (with bath at 12°C.) with the subject lying on his back in the bath. The same subject tested in still colder water (at 5.5°C.) in a vertical position (neck above water) was cooled to 32.5°C in the same exposure time. Quantitatively, the differential cooling is of the order of 300 to 400 kcal. If these results are accurate, it appears that cooling of skin areas adjacent to the central temperature regulating centers must produce either a remarkable relaxation of the peripheral vessels, or a marked inhibition of the metabolic response to immersion, or both. In the absence of such an improbable effect, the great disparity in rectal temperatures might be regarded as unrepresentative due to a relative change in rectal area circulation in the vertical and horizontal body positions. With a generally retarded circulation, such a relative change is conceivable, and might greatly alter the relation of rectal temperature to the true core temperature of the body.

Control experiments were made cooling only the neck and occiput with circulating water (at 1° to 2°C.) for periods up to three hours, the subject remaining apparently in an air environment of normal temperature. Although deep narcosis was produced in a single subject, two others experienced only a postexposure ataxia, the presence of a hyperreflexia and a positive Romberg sign being noted in all three. In no case did body temperature fall more than 0.8°C. It was noted in all experiments that the pain of cold immersion was most intense when the cold stimulus was in contact with the neck and occiput. Despite the proximity of the central temperature centers to the cooled area it cannot be said that any convincing explanation of the extraordinary quantitative effect of the local cooling of neck and occiput is available. It is probable that the effect is qualitatively real, and conceivable that the disparity was quantitatively exaggerated for the benefit of H. Himmler,<sup>1</sup> who took a personal interest in the work because of the exposure problems of S. S. troops on the Eastern front. He is reported as

<sup>1</sup> Major Leo Alexander, in his detailed report of these experiments, examined the question as to the probable validity of the data, collected under disreputable professional circumstances and with clear evidences in many places of a sadistic personality. We have also reviewed the work in detail, and agree with his conclusion, namely, that in the main, intrinsic evidence can be used to establish the probable accuracy of the report on most points.

having certain common lay convictions concerning protection of the head and spinal cord from excessive heat or cold. In any event, the point deserves investigation (under humanitarian auspices), and the reported general anesthetic effect of local neck and occiput cooling should be of interest to those who have developed local cold anesthesia as a technique in surgery on patients unfit for pharmacological anesthesia. In closing the review of this item, it must be added that a major portion of this information could have been gained without the sacrifice of human life.

*Cooling in air and water.*—The striking differences between the cooling potential of air and water of the same temperature are evident from a comparison of studies by Adolph (2) and Molnar (3), with the fatal immersion exposures reported above. In water at 1°C., sixty minutes exposure is often fatal with terminal rectal temperatures of about 25°C. (1). Unclothed exposure to an air temperature of 1°C. is tolerable for a period of at least four hours (2, 3). Heat production is increased four to five times basal. Rectal temperatures of 36°C. and skin temperatures of 15°C. are attained after one and one-half hours of exposure, and appear steady. A lower air temperature, -6°C., continued without food for fourteen hours, produces hypothermia comparable to sixty minutes at 1°C. in water (1).

Immersion studies by Spealman (4, 5) report a steady state in heat loss in water at 20°C. with a rectal temperature of 35° to 36°C. and heat production of 200 kcal. per hr. per sq. m. The same author (6) has also studied the circulation of the hand under cold, comfortable, and warm conditions. The study demonstrates that the sharp reduction in blood flow on moderate cooling of the hand is increased by greater local stress to values near those noted in warm hands. Minimum flow values of 0.9 cc. per 100 cc. per min. at bath temperature of 15°C. in contrast with rates of 4.3 at 5°C. and 5.9 at 35°C. It would be interesting to know whether or not this reversal is as marked in the digits as in the hand as a whole. Since cutaneous vasoconstriction is associated with dilation in underlying muscle (7), the practical isolation of the skin component in the digit might permit equal hand flow under warm and cold conditions but with a very different distribution of the total flow between skin and muscle.

The improbability of local respiratory damage from exposure conditions normally encountered has been demonstrated in experi-

ments giving the laryngeal and tracheal temperatures in dogs for air temperatures down to  $-50^{\circ}\text{C}$ . (8). Further observations on frost bite support the view that the fundamental lesions involve the vessels (9). Observations of the affected vessels in the rabbit by means of a transparent chamber support observations on heparinized animals indicating that the formed elements of the blood are conglomerated in the dilated vessels but not coagulated (10). Organization into thrombi requires approximately seventy-two hours, and in small frost bite lesions produced in human subjects, the subcutaneous injection of heparin limited the reaction to superficial blistering as compared with true necrosis in the uninjected controls (11).

Although no satisfactory methods of improving cold tolerance significantly or of effectively selecting cold tolerant individuals were found during the war period, most observers agree that either personal technique or natural endowment produces a great individual variability in susceptibility to frost bite, particularly under conditions of general psychic stress. In reading the report of twenty-four psychiatric examinations made by Osborne & Cowen (12) on cases of peripheral neuropathy following chilling, one is reminded of certain animals that dispense with the tail when frightened. A reviewer would not rashly assume that individuals with psychiatric complications are more susceptible to frost bite by reason of a vestigial attempt at depedalization or dedigitation under stress, but the existence of a psychosomatic factor often characterized by a prior history of vasomotor instability is apparently common, and not inconsistent with the extraordinary effects of strong emotion on digital circulation frequently observed under laboratory conditions. Studies of local vasomotor response to increased carbon dioxide in respired air and to circulatory occlusion of the limb in cases of Raynaud's disease, scleroderma, and thromboangitis obliterans have demonstrated some degree of improvement in adjustment of exposed members to cold after such treatment. (13).

A very complete investigation of the practical effect of dietary modifications on cold tolerance has been completed by Keeton and associates (14, 15). Twelve subjects, appropriately clothed, were exposed to  $-20^{\circ}\text{F}$ . for eight hours each day over a period of 5.5 months. High carbohydrate-high fat meals spaced at two hour intervals are markedly more efficient than a single high protein



meal in preventing fall of body temperature and decrement in such psychomotor functions as reaction and coordination times, speed of tapping, and flicker fusion. A full understanding of the intimate metabolic factors which may adapt a given diet to a given climatic type lies in the future. These studies, however, clearly demonstrate that there may well be a preferred dietary regimen for cold exposure. The neglect of this regimen produces a decrease in psychomotor efficiency which is surprisingly large considering the stability which these functions show to other stress conditions.

#### STANDARD THERMAL STRESS AND EQUIVALENT PHYSIOLOGICAL RESPONSE

By far the greater part of the work from the past few years now coming to general publication bears on this topic. Approximate methods of equating environments into sets of equal heat potential are widely used and large amounts of data are available for a comparative study of the problem of equivalent heat stress. It will require some time to put this material into a concise form acceptable to interested investigators. In the interim, however, investigators will be able to find in the literature what was not available in 1943, namely, one or more references reporting the primary circulatory and thermal responses to any condition between 0°C. and 50°C. in combination with full specification of the physical environment. In most instances such data are available for a number of levels of activity and in connection with many modifications of clothing, including basic data on the unclothed subject. Although not of an experimental nature, a recent publication of the Medical Research Council by Bedford (16) deserves mention in this connection because of its useful chart material. Ten large-scale interpretation and calculation nomographs relating dry and wet bulb, air movement, and radiation measurements are included in a discussion of problems of naval ventilation.

Belding, Russell & Darling (17) have compared the factors maintaining heat balance at 0°C. for subjects at rest and with various grades of activity. They have also (18) progressively undressed exercising subjects at this temperature at a constant grade of work, 300 kcal. per sq. m. per hr. The result is an interesting series showing the physiological character of successive phases of heat regulation from extreme heat stress to extreme cold. The data on circulation characteristics and successive changes in temperature gradi-

ents permit an analysis of the adjustments in tissue conductance and the associated circulatory cost. By comparing these data with those from studies under hot conditions, the independent effect of skin temperature and grade of activity on the sweat response to a given internal temperature may be realized.

Eichna *et al.* (19) conclude that acclimatization to work in heat is apparent on the second day of exposure (at 90°F. and 95 per cent relative humidity), and is complete in seven to ten days. Rest in heat confers little acclimatization to work under the same conditions. The same group (20, 21) has demonstrated that there is a difference between "tolerance" as interpreted by the soldier and the civilian. In an excellently organized series of experiments, well acclimatized soldiers completed four hours of work (250 kcal. per man per hr.) with ease at 92.5°F. (wet bulb), a condition some 4.5°F. (wet bulb) above the long quoted Haldane limit. At least three subjects completed this work at 96°F. (wet bulb). If these data are used for comparison with treadmill experiments, it should be noted that the skin temperatures are representative of the short rest period, and were probably slightly different during the march, particularly for conditions with a dry bulb greatly in excess of body temperature. In a further study (22) the effect of added clothing on the tolerance for heat has been analyzed.

Robinson, Turrell & Gerking (23) and Robinson & Gerking (24), in a series of 212 treadmill experiments over the range from 23° to 50°C., have provided the widest available range of information on the gradations of regulatory response to the different stresses imposed by differential clothing, activity, and atmosphere. From these results, *Ep*, an index of physiological effect has been computed. Increments in heart rate, rectal temperature, skin temperature, and rate of sweating over exercise values for these variables in a cool environment are given equal weight in the index. Subjects walking in shorts (producing 188 kcal. per sq. in. per hr.) were able to maintain thermal equilibrium for six hours at 34°C and 91 per cent relative humidity, with an air movement of 55 meters per minute. A 16°C. increase in air temperature was tolerable provided relative humidity was reduced to 21 per cent. This study is especially valuable since its long work period approximates the civil work day, and the results consequently have a close relation to industrial exposure problems. Such an index might gain wide acceptance if the increments of physiological strain were ex-

pressed in standard statistical measures or the insignificance of such treatment demonstrated.

Taylor & Marbarger (25, 26) have extended their studies of temporary tolerance to hot conditions up to 65°C., which are ultimately beyond the limits of adjustment. Their study concerns the sitting resting subject only. In contrast to the Robinson group above they used a statistical approach to the problem of combining physiological signs of heat stress in a single index and further concluded that sweat loss could not be included in such an index as it varied with humidity conditions independently of the degree of hyperthermia.

To date no one has attempted to incorporate the solar load in a unitary environmental temperature, although Blum (27) estimates this load at two to three times basal heat production and about equal to the marching metabolism. On this point the physiologists are somewhat behind the engineers whose sol-air temperature (28) index combines solar and atmospheric heat loads. Studies of the biophysical constants of heat loss have made other substantial progress, however, in the analysis by Nelson, and co-workers (29) of the physically complex relation of human heat loss to air movement. The interrelations of evaporation, radiation, and convective heat loss from an object that can elect, to some degree, its mode of response, are by not means simple. These authors have studied air velocities ( $V$ ) from 30 to 600 feet per minute in seven hot environments on resting and exercising subjects. Heat loss by evaporation ( $E$ ) is given as

$$E/\Delta P = 1.4V^{0.4}$$

where

$E$  is in kcal. per hr. per sq. m.,

$\Delta P$  is vapor pressure difference between skin (saturation pressure at measured skin temperature), and air in mm. Hg.,

$V$  is in feet per minute.

The radiation loss,  $R$ , and convection loss,  $C$ , in kcal per hr. per sq. in. is reported as

$$(C+R)/\Delta T = 5.6 + 0.53V^{0.5}$$

where

$\Delta T$  is the difference between air and skin temperatures in °C.,

$V$  is the velocity in feet per minute,

and where the equation applies to conditions of equal air and wall temperature.

Shelly & Horvath (30) have reported data on sixteen soldiers progressively acclimatized to work requiring a total heat production of 250 kcal. per hr. in an environment of 120° (dry bulb), and 93° (wet bulb). Tolerance for this severe condition improved from 0.5 hour on the first exposure to 1.6 hours on the fourteenth day. The same authors (31) have contributed data on the relation of oral and rectal temperatures under heat stress which are useful in the analysis of many field and industrial reports which note only the oral temperature. Information on the comparative values of shallow and deep rectal temperatures would do much to reconcile the data of Nielsen (32) with war period studies in which this measurement was necessarily taken with less elaborate care. Nielsen's careful study showed no reliable change in deep rectal temperature in the same work performed at air temperatures from 5° to 36°C. The point is of more than passing importance since a vast amount of laborious experimentation has resulted from the war, all of which is available for the construction of a stress index provided that the primary thermal measurements can be related to a common technique.

In centrifuge experiments, Code *et al.* (33) have shown that an increase in environmental temperature (from 63°F., 72 per cent relative humidity to 89°F., 77 per cent relative humidity) decreases "g" tolerance by 0.7 to 0.9 g. This is further proof of the fact that heat stress reduces the effectiveness of vasomotor compensations for postural change.

#### SWEAT, BODY FLUIDS, AND METABOLITES

Search for an explanation and remedy for the presumed debilitating effects of the tropics on the white man has ranged over a field including social alcohol, absence of the necessity for physical activity, and what might be called cultural inhibitions in adopting native habits of adjustment. More recent recognition of the possible loss of considerable amounts of water soluble dietary essentials in sweat has made it apparent that if such losses represent more than a diversion of excretion from kidney to sweat glands, they might have an important bearing on tropical nutrition. A number of studies directed to this problem have recently appeared from the Division of Animal Nutrition at the University of Illinois.

The relative excretions in sweat and urine of ascorbic acid, dehydroascorbic acid (34), pantothenic acid (35), inositol, *p*-amino-

benzoic acid (36), folic acid (37), nicotinic acid, nicotinamide, nicotimuric acid, *N I* methyl-nicotinamide (38), pyridoxine, "Pseudopyridoxine," 4-pyridoxic acid (39), choline (40), and iodine (41) have been reported. All of these substances were found in the sweat of subjects exposed to a hot moist environment. In no case did the authors find unequivocal evidence that the total loss in sweat, or in sweat and urine combined, was increased by amounts likely to be of dietary importance. However, the conclusions are conservatively reported, and it is noted that the total dermal and urinary excretions of pantothenic acid and ascorbic acid are increased somewhat in hot moist conditions. In general, only negligible amounts of the studied nutrients were found in the dermal fraction; however, folic acid proved to be an exception, the ratio of the sweat to urine fraction being between five and six. The total amount, however, is not large in relation to the requirement of 0.5 to 1.0 mg. units suggested by Johnson (37).

Johnston, Conn & Lewis (42) have shown that under conditions of hard work and humid heat, the hand sweat provides a representative sample of the total 5 to 10 liter sweat secretion in so far as chloride and nitrogen balances studies are concerned. The same team (43) has reported marked individual differences in the ability to establish a balance on low salt intakes and a maximal individual variability in sweat chlorides on a high salt intake. Moreira *et al.* (44) have found that large doses of adrenal cortical extract have no beneficial effect on working capacity in heat. Potassium is increased in the urine, and the sweat concentrations of sodium, potassium, and chloride may be altered. Gerking & Robinson (45) reported a decline in rate of sweating at high rates of secretion during prolonged exercise (six hours on a treadmill), the decline being distinctly greater in humid environments in contrast to dry heat when initial rates were comparable. A technique for enumeration of functional sweat glands by means of starch-iodine impregnated tissue has been used in a comparison of the relative secretion response to acetyl-beta-methylcholine and heat stimulation (46).

Adolph (47) concludes that the promptness of onset of sweating is no measure of individual heat tolerance, and that the stimulus required for sweating is an appreciable increase in the heat content of the body as a whole. With the inclusion of additional measurements, further studies of this type should be rewarding, despite the negative result on tolerance discrimination. It appears highly prob-

able that the steady state in sweat secretion is maintained by at least three factors: skin temperature, internal temperature, and a central excitability related through reflex or humoral mechanisms to the level of activity. In any event, the level of sweat secretion cannot be predicted from rectal temperature. Compare Nielsen (32), Robinson (23, 24), and Belding's (17, 18) work on temperature regulation and other studies of the mechanism of respiratory control during muscular work (48 to 51).

Further studies have been made of the water balance and heat balance of the respiratory tract (52, 53, 54) under different climatic conditions, and of water balance in man as affected by temperature (55) and fluid intake (56, 57), and in the rat in response to changes of vapor pressure at constant temperature (58). It is again noted that the temperature of the environment is an important variable in animal survival of traumatic shock (59 to 63), and that restoration of the normal temperature in the extremities of epinephrine-shocked animals is not beneficial (64). The adult rat is surprisingly susceptible to local overheating, immersion of the extremities at 45°C. for two and one-half hours resulting in death attributed to heat injury and a toxic factor (65). This susceptibility is acquired with age, since the newborn rat is very tolerant of temperature change, survival being noted over a body temperature range from 20° to 43°C. (66). In much of the work on animal shock in which environmental temperature or heat applications are reported in an accessory but important role, experimenters seem careless of a very old but fundamental fact, namely, that animals of greatly different size and metabolic intensity respond very differently to physically standard heat or cold exposures applied for a standard time. Kleiber's (67) report on the fifteen-fold variation in the survival time of starving rats between 30° and 38°C. should call to mind, for these experimenters, the dynamic effect of temperature variation on small animals. Brobeck's (68) study of temperature effects in relation to activity, weight gain, and food intake in the rat provides a clear example of the sensitivity of these small animals to environmental changes which are of greatly reduced importance in man and the larger experimental animals. In the same vein one might quote the conflicting findings on the requirements for thiamine (69), choline (70), and other essential nutrients (34 to 41), as affected by environmental temperature. Much confusion results from the extension to man of data obtained on animals whose me-

tabolism is highly sensitive to even a few degrees change in temperature. In the rat or mouse an increase of 40 to 50 kcal. per sq. in. per 24 hr. occurs for a shift in environmental temperature of 1°C. in either direction from the critical temperature of 28° to 29°C. In man it is difficult to detect any change for the range of habitable temperatures.

#### CLIMATOLOGY AND CIVILIAN PROBLEMS

In the war period, man in the mass discovered that the entire spectrum of climates is available only a few miles up or a few hours away. Perhaps twenty million men experienced climatic exposures, heat stresses, and activity limitations unknown in their native habitats. It is generally agreed that we are now entering a period in which problems of acclimatization will assume a new importance determined by the developments of rapid transportation facilities and the existence of widespread pressure for population migration (71). Both the human and animal aspects of climatic adaptation will have an unparalleled economic importance. In many respects Australia is a natural laboratory for the incubation of these problems, and in that location we find active concern and every evidence of scientific progress. A number of publications have appeared (72 to 78) from the laboratory of D. H. K. Lee at the University of Queensland, on the temperature susceptibility of various animals of economic importance in colonization. The same investigator and his colleagues have made a number of war-period studies on human psychomotor adjustment under heat stress which have not yet been generally released.

Two recent papers emphasizing the concern with tropical debility have dealt with instances in which appetitive mechanisms in newcomers to the tropics have apparently failed to adjust the individual to an altered fluid exchange. Rose reports that crystaluria and calculi are particularly common in newcomers (79). He is of the opinion that an initially satisfactory stimulation of fluid intake is gradually replaced by tolerance for a reduced transfer with resulting greater concentration of urine, and that this tendency is not evident in those of tropical birth. A gradually developing chronic hypochoremia simulating psychoneurosis, which responds readily to vitamin and salt supplements, has also been observed in tropical newcomers (80). Disorders due to heat are reviewed from the standpoint of therapy by Park (81). The condi-



tioning of hospitals and operating rooms both in the tropics and in the temperate zone must take account not only of the apparent comfort of the patient but of the problem of acclimatization as presented by the patient's transfer from room quarters to operating quarters and *vice versa* (82). Preoperative patients should not be held in air-conditioned quarters unless the operating room is similarly treated.

The peculiar behavior of certain epidemic diseases in respect to seasonally conditioned changes of temperature is well known. Recent studies (83) on the effect of environmental temperature on mouse susceptibility to poliomyelitis virus indicate that there is a progressive reduction in incubation period and the period for a standard percentage mortality as environmental temperature is raised from 13°C to 32°C. It is suggested that in human beings the epidemic subsides in the Fall due to a stimulated host metabolism which lengthens incubation and permits the development of resistance. If this effect is primarily a matter of intensity of metabolism rather than some feature of the associated thyroid-adrenal stimulation, rats at 35°C. should prove as resistant as those at 13°C.

In view of the remarkable effects of temperature on industrial production and the subjective prominence of lassitude in heat exposure, strikingly little progress has been made in demonstrating temperature influenced changes in psychomotor efficiency. Weiner & Hutchinson (84) report that work in environments above 88° Effective Temperature results in a deterioration of performance in motor coordination tests. Kleitman's (85) study of body temperature during motion picture attendance, while not directed at climatic factors, clearly shows that psychic stimulation has a large and readily detectable effect on temperature regulation evidenced in body temperature increases of 0.5° to 1°F.

In a general discussion of the fitness of various body structures in relation to temperature adjustment, Cowles (86) makes a good case for the initial appearance of fur and feathers as a mutation initially favorable to the protection of poikilotherms from solar radiation; the later advantages incident to the development of homeothermy being secondary. Several papers (87, 88) have recently appeared on the effect of temperature gradients on the intensity and duration of traumatic pain, and Kleitman (89) has added a study on cutaneous sensitivity to pain to the long list of physiological functions whose activity he has related to general

body temperature. Swift (90) has re-examined the effect on the critical temperature of the rat while under fast and while receiving a diet characterized by a high dynamic effect. The zone of thermic neutrality for the fasting rat was found to be from 28° to 33°C. The effect of feed being to lower both limits of this range by 1°C. Galvão (91) has reported the heat production of dogs ranging in weight from 3 to 31 kgs. He concludes that, in an environment of moderate warmth (18°C.), the intraspecific metabolism of the dog is approximately constant per unit of weight if a wide range of absolute weights is compared. If compared on a basis of surface area, heat production increased with absolute size. All determinations were made on anesthetized animals. Although the effect of the anesthesia was discounted by a control series, the results would be of greater value had they been obtained on the unanesthetized animal.

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AERO MEDICAL LABORATORY  
WRIGHT FIELD  
DAYTON, OHIO  
JOHN B. PIERCE FOUNDATION  
YALE UNIVERSITY SCHOOL OF MEDICINE  
NEW HAVEN, CONNECTICUT

## THE RESPIRATORY SYSTEM<sup>1</sup>

BY HAYDEN C. NICHOLSON

*Department of Physiology, University of Michigan,  
Ann Arbor, Michigan*

During the past year there has appeared a large volume of literature dealing with the physiology of respiration, much of it of unusual significance. Relaxation of security regulations finally has permitted the reporting of numerous investigations dealing with matters of military importance and is responsible for the considerable emphasis in this review upon anoxia and other aviation problems.

### ANOXIA

Blood, Elliott & D'Amour (1) have made a very comprehensive study of the physiology of the rat in extreme anoxia. At forty thousand feet oxygen consumption decreased 57 per cent. Body temperature fell 3.2° C. Other data indicated a liberation of carbon dioxide due to acidosis. Britton & Kline (2) determined the resistance to anoxia of numerous small animals. Rat fetuses and immature pouch opossums were very resistant. Adult female rats were more resistant than males, a difference attributed to the larger adrenal cortex in the former. Fully fed animals survived longer than fasting animals. Glucose increased and insulin decreased survival time.

Arshavski (3) presents evidence indicating need for reevaluation of the commonly accepted view that young animals are much more resistant to anoxia than adults. He found that in dogs death from anoxia is preceded by an abrupt drop in blood pressure. As judged by the time to onset of this "shock" stage, adult animals are much more resistant to anoxia than are young, this increase resulting from the development of the carotid sinus and cardio-aortic reflexes. In the adult dog subjected to progressive lowering of the oxygen in the inspired air, "crisis" occurs when the oxygen falls below 4 per cent and cardiac arrest and death follow within a minute. In puppies the crisis, characterized by an abrupt drop in breathing to two to three per minute and in arterial pressure to zero, sets in when the oxygen has fallen only to 14 to 15 per cent. The heart may continue beating slowly for an hour. Cardiac

<sup>1</sup> This review covers the period from July 1, 1945 to June 30, 1946.

automatism of the new born and young animals nearly coincides with that of the cold blooded heart. The respiration persisting is spinal.

Fender, Neff & Binger (4) subjected bitches late in pregnancy to severe anoxia. Of the five pups surviving the first few weeks, two developed status epilepticus. It is suggested that fetal or neonatal anoxia may play a major part in the development of epilepsy in man. Barcroft & Young (5) artificially prolonged gestation in rabbits. Fetal anoxia, from continuation of fetal growth after cessation of placental growth, presumably accounted for the intrauterine respiratory movements and ultimate death of the fetus. Windle, Jensen and their co-workers (6 to 9) exposed guinea pigs to simulated altitudes of twenty-three and thirty thousand feet, six hours a day. Significant increases in hemoglobin, red cells, reticulocytes, spleen weight, and hematocrit were observed after one hundred hours' exposure. The effects were doubled after two hundred hours, with no further increase beyond that period. There was increasing memory loss after one hundred and fifty, two hundred, and two hundred and fifty hours at thirty thousand feet. Animals exposed to twenty-three thousand feet for as long as five hundred hours showed no histopathological brain changes. Morrison (10) found that exposure to thirty-two thousand feet for twenty-five minutes produced extensive laminar necrosis in the cerebral cortex of the monkey. With repeated exposure to mild anoxia, the first changes occurred in the cell bodies of the cortical gray matter. This took place at a level of twelve to thirteen volumes per cent oxygen in the blood, if the exposures were long and frequent enough. When the oxygen was reduced to ten volumes per cent, and the number of exposures increased, the white matter also became involved. The adrenals showed evidence of increased cortical activity.

Stickney (11) found that intermittent anoxia (four thousand to eighteen thousand feet) caused weight loss in rats roughly in proportion to its severity. Nims *et al.* (12) report that anoxia increased liver glycogen in rats, an effect they attributed to the induced hypocapnia. Hollinshead (13) found that severe anoxia in mice resulted in marked degranulation of the cells of the carotid body, suggesting that the granules of those cells are concerned with the initiation of chemoreceptor reflexes. Van Harreveld (14) observed during asphyxiation of the spinal cord that the gray mat-



ter became negative with respect to the outside of the cord, an effect which he interprets as an expression of the depolarization of the parts of the neurons most sensitive to oxygen lack. MacLachlan (15) found that anoxia caused an initial acceleration of the emptying of the rat's stomach. Hartiala & Karvonen (16) found in human subjects that the depression of gastric hydrochloric acid secretion by anoxia occurred regardless of a previous administration of ammonium chloride and, hence, was due to the anoxia *per se* and not to the alkalosis from hyperventilation.

Arellano Z (17) found cerebrospinal fluid pressures in human subjects averaging 2.6 cm. higher at 4,538 meters than at sea level. Individuals acclimatized to one thousand and to six thousand two hundred feet were exposed by Boothby (18) to decreased barometric pressures in the low pressure chamber, the latter group showing increased resistance to anoxia. Hurtado, Merino & Delgado (19) studied the effects of high altitude on the blood of healthy and diseased men. There was observed a polycythemic response resulting from erythropoietic hyperactivity and proportional to the degree, duration, and continuity of the anoxic stimulus. It appears that in cases of anoxemia at sea level due to pulmonary changes, the polycythemic response is less than with corresponding degrees of arterial unsaturation at high altitudes, except in cases of Ayerza's disease. It also is concluded that the causative mechanism of polycythemia vera is not related to anoxia. Lurie (20) found in healthy men and women acclimatized at 5,740 feet mean red cell counts of 5,593,000 and 4,999,000, respectively, with proportional elevation of the hemoglobin.

Henry *et al.* (21) studied the effect of anoxia on capillary permeability of the human arm. Effects of thirty minutes of venous congestion were not significantly different at twenty thousand feet from those at sea level. Anderson and co-workers (22) investigated circulatory changes during anoxic fainting and coma, comparing the effects of 7 to 8 per cent oxygen with 7 to 8 per cent oxygen plus a simulated hemorrhage produced by occluding the venous return from the thigh. Of thirteen subjects, three fainted during hypoxia while ten fainted during hypoxia plus simulated hemorrhage. These observations emphasize the increased danger from anoxia in individuals who have lost blood. Harris (23) found in dogs that clamping a coronary artery frequently caused ventricular fibrillation, while generalized anoxia almost invariably resulted in

ventricular standstill instead. Baum *et al.* (24) compared the effects of exercise and anoxia upon the human electrocardiogram. Engel, Webb & Ferris (25) observed significant changes in frequency distribution of the electroencephalogram at a simulated altitude of ten thousand feet. Sugar (26) from an electroencephalographic study of cadets fainting at eighteen thousand feet in the low pressure chamber concluded that the syncope represented a psychosomatic, rather than a basically epileptic, disorder.

How & Duff (27) exposed fifty subjects with recent history of malaria to a simulated altitude of eighteen thousand feet for one hour. None relapsed within seven days, or showed a positive blood smear within five days. Rostorfer & Rigdon (28) found a diminished resistance to anoxia in ducks and chicks infected with malaria.

Adler (29), studying the effects of anoxia on heterophoria, observed a shift of the range of fusion toward the convergent position. Hartline (30) reviewed the effects of anoxia on vision.

Beyne & Michelle (31) found that in normal young adults respiratory acceleration began at a simulated altitude of twelve hundred meters (in one subject sixty-three years old, at four to seven hundred meters). D'Angelo (32, 33, 34) exposed men to simulated altitudes of eight and ten thousand feet for ten hours. He observed changes in respiratory metabolism indicative of an alkalosis, no change in blood sugar or urinary output, but a significant decrease in excretion of inorganic phosphorus. Greeley *et al.* (35) found that breathing 11.5 per cent oxygen in nitrogen did not decrease "g" tolerance significantly. Kirsch (36) reports increases in pulse rate, blood pressure, respiration, and perspiration in aviators during combat flights. Preston (37) presents a series of one hundred and thirty-two children in whom anoxia due to various prenatal and postnatal causes damaged the central nervous system seriously enough to affect subsequent behavior.

Hoffman *et al.* (38) studied the duration of useful consciousness at various simulated altitudes and reported durations slightly less than those of MacKenzie and co-workers (39).

The need of an impressive demonstration of the early effects of anoxia was evident to personnel in the altitude training programs of the services. Fulton (40) and Rodbard (41) reported the effects of variations in simulated altitude upon breath holding. Marzulli (42, 43) upon the ability to receive radio code, Shaklee

*et al.* (44) upon scores made with a synthetic gunnery trainer, and Birren *et al.* (45) upon critical flicker frequency, perimetry, and body sway. Ricketts and co-workers (46) report that while working in the low pressure chamber at simulated altitudes of ten thousand and eleven thousand five hundred feet, they all experienced definite impairment in muscular coordination, efficiency, memory, and clarity of thinking. Sleepiness, irritability, fatigue, and loss of initiative were common, and unusual concentration was necessary in making observations. While subjective observations are notoriously unreliable in this field, when one takes into account the experience of these investigators and considers their observations in conjunction with numerous reports from aviation personnel, the conclusion becomes inescapable that individuals exposed to altitudes of ten thousand feet without supplementary oxygen are functioning well below peak efficiency.

During the war a large number of persons assigned to the altitude training programs of the Army and Navy were exposed at frequent intervals to simulated altitudes as high as thirty-eight thousand feet. Bricker (47, 48) analyzed 1174 physical examinations on 461 altitude chamber technicians in the A.A.F. The only significant finding which apparently could be attributed to low pressure chamber experience was a higher incidence of clouding in the accessory sinuses. McMichael & Goggio (49) attempted to evaluate, subjectively and objectively, the mental status of 140 altitude chamber technicians. They found no evidence of mental deterioration. If one may assume that the physical and mental examinations used were sufficiently reliable and sensitive, it may be concluded that these men were protected in their work by reasonably adequate safeguards.

*Resistance to anoxia as affected by various factors.*—Green, Butts & Mulholland (50) and Eckman *et al.* (51) describe an increase in altitude tolerance from a high carbohydrate diet. The latter workers found that such a diet compared to a high protein-fat diet increased altitude tolerance approximately one thousand feet as indicated by changes in arterial blood gases, thirteen hundred feet as indicated by alveolar air composition, and one to two thousand feet as indicated by psychological and psychomotor tests. The gain they ascribe to the increased respiratory quotient. No effect would be expected at altitudes high enough to require the breathing of pure oxygen. McFarland and co-workers (52) describe the use of

differential intensity thresholds for vision at low brightness levels as an extremely sensitive objective index of functional impairment by anoxia. They found that ingestion of fifty grams of glucose by fasting men lowered the "physiological altitude" 25 to 48 per cent. Smith & Oster (53) found the effects of starvation upon resistance of cats to anoxia to be variable. Insulin decreased resistance to anoxia. Heistand *et al.* (54, 55) found that vasopressin increased the survival time of the primitive respiratory center in the isolated rat head, and increased the blood sugar, while oxytocin had no effect on either. Effects of epinephrine, ephedrine, insulin, and glutathione are also described. Charipper and co-workers (56, 57) found that rats whose diet was restricted in amount, or deficient in vitamin B, showed increased resistance to anoxia, an effect counteracted by administration of thiamine. Thiourea and thiouracil increased resistance to anoxia, while estrogens were without effect. Thorn *et al.* (58) observed increased resistance of rats to anoxia from adrenal cortical extract, but not from desoxycorticosterone, 17-hydroxycorticosterone, or sodium succinate. Barach and co-workers (59) studied the effects of ingestion of ammonium chloride on altitude tolerance in men. At all altitudes the alveolar carbon dioxide tension was depressed, and the oxygen tension elevated. Subjective sensations of altitude were markedly lessened or abolished. Brooks (60) found some evidence of improved efficiency at altitude from administration of methylene blue. Snyder (61) found that in newborn rabbits sodium pentobarbital increased the duration of respiratory movements following tracheal ligation. Van Liere & Marsh (62) studied the effects of ergotamine, yohimbin, 933 F., epinephrine, ephedrine, and cocaine upon the anoxic rise in blood pressure in dogs. In thirty-one aircrew candidates with chest deformities, Ratnoff (63) could find no evidence of decreased resistance to anoxia. Kalk & Brühl (64) found decreased resistance to anoxia in mild fever, infections, exudative pleuritis, hyperthyroidism, secondary and pernicious anemia, cachexia, valvular heart disease and fatigue, with increased tolerance in some liver diseases and allergies.

*Carbon monoxide.*—Lilienthal and co-workers (65, 66) found that the rate of uptake of carbon monoxide was inversely proportional to the partial pressure of oxygen, the total barometric pressure being without effect. Using critical flicker fusion frequency as a measure of functional impairment they found that increments of

carbon monoxide hemoglobin of the order of 5 to 10 per cent resulted in appreciable deterioration at altitudes (five thousand to six thousand feet) which alone were without obvious effect. Tobias *et al.* (67), using radio-active carbon monoxide, could find no evidence that carbon monoxide was converted to carbon dioxide in the human body. Roughton & Root (68) found that in normal men breathing oxygen or air, the carbon monoxide in the expired air averages only 60 to 70 per cent of that currently lost from the blood during the first hour after the carbon monoxide administration. If, however, the subjects continue to breathe oxygen for four hours after the carbon monoxide administration, about 96 per cent of the carbon monoxide initially absorbed is recovered in the expired air. It is inferred that the 30 to 40 per cent lost from the blood in the first hour, and unaccounted for in the expired air, must have combined reversibly with hemoglobin-like pigments outside the main blood stream. The experimental results indicate that there is no significant loss of carbon monoxide through the skin, sweat, urine or feces, or by oxidation or other forms of metabolism, at least in mild carbon monoxide poisoning. Dévoir, Truffert & Derobert (69) discussed chronic carbon monoxide poisoning in man. Lewey & Drabkin (70) found that dogs exposed for five and a half hours a day, six days a week for eleven weeks, to an atmosphere containing 0.01 volumes per cent carbon monoxide and reaching daily 20 per cent carbon monoxide hemoglobin, showed a constant disturbance of postural and position reflexes and gait. Some showed a pathological electrocardiogram characteristic of anoxia, as well as necrosis of single heart muscle fibers. The central nervous system at examination three months after the end of the experiment showed characteristic histologic changes. The findings indicate that carbon monoxide intoxication may occur in dogs at concentrations which have been regarded as within the limits of safety for man. Pappenheimer (71) discusses the carbon monoxide hazard in aircraft.

#### OXYGEN CONSUMPTION

Taylor (72) found that six months of semistarvation was without effect on oxygen consumption per gram of active tissue. Bronk and co-workers (73, 74), using the oxygen electrode devised by Davies & Brink, obtained some very significant data on the oxygen consumption of the cerebral cortex and sympathetic ganglia of

cats. It was found that following interruption of the blood flow to a localized cortical area, the oxygen tension falls linearly until it reaches a value of 2 to 3 mm. Hg., indicating that above this level the rate of oxygen consumption is independent of tension. The high rate of cortical metabolism is indicated by the fact that the tension falls to this level in from one to ten seconds, contrasted with ninety seconds in sympathetic ganglia. It was found that when one traverses the cat cortex with the oxygen electrode there are encountered gradients of oxygen concentration corresponding to the pattern of the vascular bed, the tension varying between 100 mm. Hg and near zero. Consequently one cannot speak of an "oxygen tension of the cortex" for each region has its special value.

Other papers discuss the following: effects of pharmacological agents on cerebral oxygen tension (75), pilot size in relation to oxygen cost of piloting aircraft (76), and apparatus and methods to be used in field studies of work animals (77, 78).

#### HIGH OXYGEN PRESSURES

As has been true for some time the two groups most active in the investigation of the effects of high oxygen have been Bean and his collaborators at Michigan, and Stadie, Riggs and their co-workers at Pennsylvania. The former group (79 to 83) described acute and chronic motor disability in rats and chicks and memory loss in rats as the result of exposure to high oxygen pressures. Histological examination showed widespread degenerative changes in the central nervous system. It is the view of these workers that the effects represent a combination of increased carbon dioxide tension in the tissues, increased tissue acidity, and a direct action of high oxygen on cellular enzyme mechanisms. Porter & Bean (84) showed a marked adverse influence of increased oxygen pressure on malarial parasites. Riggs (85) reports experiments confirming the observations of Bean & Bohr that high oxygen depresses the tonus of the pyloric sphincter, and that this loss of tone is prevented by cyanide. However, on the basis of the observation that high oxygen did not affect significantly the oxygen uptake of the sphincter, he questions the suggestion of Bean & Bohr that the tonus change is due to hypoxic anoxia from inactivation of the dehydrogenase system. Stadie *et al.* (86 to 91) studied the effects of high oxygen upon the metabolism of brain, liver, kidney, lung and muscle tissue, and upon various enzyme systems. Gersh (92,

93) presents evidence that the cerebral cortex is the site of origin of oxygen seizures.

Comroe *et al.* (94) found that 100 per cent oxygen administered to normal men continuously for twenty-four hours produced substernal distress in 82 per cent, usually with a significant decrease in vital capacity, and frequently accompanied by signs of nose and throat irritation. He recommends, in agreement with the views expressed by Bean in his recent review, that when oxygen must be given for a period in excess of twelve hours, the concentration should be reduced to 60 per cent; if the condition of the patient demands a higher percentage, a careful check should be made for the symptoms most likely to occur. Whitehorn, Edelmann & Hitchcock (95) administered pure oxygen to normal men and observed a lowering of cardiac output as determined ballistocardiographically. Kaunitz (96) exposed mice to 100 per cent oxygen at atmospheric pressure for seventy-two hours. Most of the animals died during this period. Examination of the respiratory tract showed changes comparable to those found after exposure to phosgene. Death of the animals is attributed to oxygen want.

#### DECOMPRESSION SICKNESS

Harvey (97) reviewed the subject of decompression sickness, and summarized the methods and results of his very significant studies relating to the mechanism of bubble formation and its role in the production of bends. Whiteley & McElroy (98) studied the removal of nitrogen from various tissues of the anaesthetized cat, their results supporting their view that the tissue concerned with bubble formation in the exercising cat is skeletal muscle. Hetherington & Miller (99) injected nitrogen intravenously in anaesthetized cats, observing its effects upon various respiratory and circulatory functions. Gersh, Catchpole *et al.* (100 to 103) investigated extensively the factors affecting the formation and distribution of gas bubbles in rabbits decompressed from high and from normal atmospheric pressure. McArdle (104) found that the elimination of nitrogen from the cerebrospinal fluid of human subjects was slow, but not as slow as from fat.

Lund & Lawrence (112) found that in patients in whom bends in elbow, knee, or ankle had been relieved by increasing the atmospheric pressure, milking the limb toward the original site of pain might reinduce or increase the pain or shift it closer to the



joint ahead of the massaging hand. These observations are interpreted as supporting the view that there occur collections of gas in the fascial and intermuscular septal planes which cause pain by dissecting to the periosteal insertions of such anatomical layers.

Henry (117, 118) discusses altitude pain in relation to exercise, distribution, time of onset, predictability, and other factors. He found that the incidence and severity of the pain are related to total work in foot-pounds, rather than to muscle strain, mechanical tension, or amount of joint movement. The results are interpreted as supporting the view that increased carbon dioxide production is principally responsible for the effects of exercise on the occurrence and severity of altitude pain. Motley, Chinn & Odell (119) studied the incidence of bends in 68,422 aircrew trainees given simulated flights in the low pressure chamber. Bends incidence was highest in the morning, lower in individuals exposed to a brief period of anoxia, higher in older and larger subjects, over twice as high in individuals who had failed to complete a previous flight as in unselected subjects, and higher in the same location on successive flights. Guest (120) and Ray (121) also report bends incidence highest in the morning. The latter worker analyzed bends and chokes incidence in relation to time.

Fulton & Phillips (122) analyzed the symptoms causing forced descent in a group of thirty-six thousand individuals given low pressure chamber "flights." Sudden dizziness, syncope, and collapse, with no discernible pain, occurred in 0.2 per cent of all trainees ascending to thirty-eight thousand feet for fifteen minutes. Of this number 12 per cent also fainted during a flight to eighteen thousand feet without oxygen, only 0.62 per cent of an unselected group collapsing during such a flight. Marzulli (123) found that of the syncopal reactions occurring in subjects exposed to simulated altitudes of eighteen or twenty thousand feet for twelve or fifteen minutes, 62 per cent occurred in the first five minutes. Brown (124) found that a considerable proportion of the cases of syncope in low pressure chambers occurred in the absence of pain or other obvious cause. Griffin *et al.* (125) found that increased speed of ascent increased bends incidence, even after elimination of the preoxygenation factor. Lawrence *et al.* (128) found that with similar rate of ascent, altitude, and temperature, the incidence of bends was the same in the airplane as in the low pressure chamber. Smith (129) reports that morphine is effective in relieving decompression pain,

but when given subcutaneously its action is too slow to make it of practical use. Barach *et al.* (130) describe two cases of activation of apparently quiescent pulmonary tuberculosis in physicians working in the low pressure chamber, which they believe is the result of liberation of bacilli by gaseous expansion and rupture of an old tubercle.

Additional reports are given on decompression sickness and bends in relation to the following: descent from high altitudes (105, 106, 108), diving operations (107, 108), "chokes" in high altitude flying (109), bone lesions in submarine personnel (110), traumatic calcifications (111), relief by arterial compression (113), control by preselection and by denitrogenation (114, 115), prevention by preoxygenation (116), effect of environmental temperature on incidence (125, 126), and relation to physical fitness (127). A number of papers (131 to 136) deal with the effects of rapid decompression in man and animals.

#### EFFECTS OF CARBON DIOXIDE

Stone *et al.* (137) found that the effects of hypoxia upon various chemical constituents of cerebral tissue were diminished by carbon dioxide. Petrov (138) found that carbon dioxide increased the survival time of mice and young rats exposed to 2.5 per cent oxygen. Garasenko (139), using changes in urine chemistry as criteria of severity of anoxia, found that carbon dioxide had a definitely beneficial effect. He suggested the use of oxygen-carbon dioxide mixtures in aviation, but concluded that his experimental data at present do not warrant the routine use of such mixtures. Gray (140) constructed tables which supply much pertinent information regarding the use of carbon dioxide to counteract anoxia. He concludes (a) that replacement of inert gas by carbon dioxide ameliorates the anoxia of medium altitudes, but less effectively and less economically than by equal percentages of oxygen; (b) that replacement of oxygen by carbon dioxide aggravates the anoxia of high altitudes, and because of the hyperpnea interferes with the economic use of the oxygen supply; (c) that the use of carbon dioxide in military aviation is inadvisable. A number of papers dealing with this subject have come from Fenn's laboratory (141, 142, 143). A new method of representing alveolar air concentrations at altitude is described. The various curves plotted depict with considerable clarity the relationships between carbon dioxide and

oxygen pressures under various conditions. Performance tests lead to the conclusion that while acapnia and anoxia effects tend to be additive, performance impairment at altitudes up to twenty-two thousand feet is essentially a matter of anoxia alone. In experiments involving artificial ventilation at a simulated altitude of thirty thousand feet, it was found that hypocapnia had little effect upon the performance until the alveolar carbon dioxide tension fell below 25 mm. Hg.

Gibbs *et al.* (144) found that hyperventilation reduced and carbon dioxide increased cerebral blood flow in man, a finding supported by the observations of Kety & Schmidt (145, 146). Christensen (147) found that hyperventilation caused a constriction of the larger arteries of the extremities as indicated by oscillometric determinations. Mayerson (148) found that 4 to 7 per cent carbon dioxide tended to prevent postexercise orthostatic circulatory insufficiency. Beckman (149) found that poor visibility and heavy traffic markedly lowered carbon dioxide combining power in aviators, these observations emphasizing the importance of apprehension as a cause of the hyperventilation syndrome. Lederer & Kidera (150) describe three cases of hyperpneic tetany occurring among commercial aircraft passengers. Van Middlesworth & Britton (151) found that carbon dioxide diminished the blood pressure drop which in monkeys and dogs results from exposure to acceleratory forces. Gernandt & Zotterman (152) found that the injury potentials in the afferent splanchnic fibers of the cat, and pricking paresthesias in man are increased by overventilation and decreased by carbon dioxide, effects probably explained on the basis of changes in ionized calcium.

#### RESPIRATORY CONTROL

Recent work of Gesell, who has been so active in this field over the past twenty-five years, has concerned itself not with respiratory control as such, but with the application and extension of his views, originally based upon studies of respiratory mechanisms, to general problems of nervous integration. A number of papers bearing importantly upon this subject have appeared during the past year (153 to 157).

Heymans, Pannier & Verbecke (158) showed by cross circulation and other experiments that the respiratory stimulant effect of acetylcholine is largely reflex, and interpret their results as oppos-

ing the view that a cholinergic mechanism is involved in central nervous system excitations. While the observation is doubtless correct, its relevance as regards the latter point seems questionable. Boelaert (159) found that the respiratory center of *Lacerta ocellata* can be stimulated by anoxia, sodium sulphide, nicotine, and lobeline after denervation of the vascular zones homologous to the carotid sinus and cardio-aortic zone of mammals. Bucher (160, 161) found that lowering the cerebral arterial pressure to 9 mm. Hg by clamping the carotids resulted in respiratory arrest after ten seconds. Heymans (162) found that wide changes in cerebral blood pressure, or flow produced by vascular occlusions did not stimulate respiration. From these observations, he concludes that the activity of the respiratory center is determined not by its own metabolism, but by a specific action of carbon dioxide. To the reviewer, the relationship between the observations and the conclusions seems somewhat remote. Ogden (163) has made some very intriguing and significant observations on respiration in the dog-fish.

"The Multiple Factor Theory of the Control of Respiratory Ventilation," presented by Gray in *Science* (164) and in more detail in three A.A.F. School of Aviation Medicine reports (165, 166, 167) states that a number of factors exert independent effects upon respiration, the actual ventilation representing the algebraic sum of the partial effects. Gray has chosen acid, carbon dioxide, and oxygen as three factors importantly concerned in respiratory control and, while recognizing that their concentrations ideally "should be measured in the respiratory center and the peripheral chemoreceptors where they exert their effects," has utilized their arterial concentrations in the derivation of four equations, which predict with some accuracy the respiratory volume in various situations where these three factors predominantly are involved. In his discussion, Gray refers to preceding theories, apparently including those of Nielsen and Gesell, as "single factor theories" to which his "multiple factor theory" stands in contrast.

While Gray's articles are exceedingly thought-provoking, the reviewer has great difficulty in finding there a new theory of respiratory control. Dr. Hugo Krueger, in response to a personal request to evaluate the paper in *Science* from a mathematical point of view has commented upon Gray's neglecting to accompany his chemical ventilation equation with a statement of standard error

or with a statement of the limitations defining the conditions under which the equation can validly be used. Krueger emphasizes that such a prediction equation does not offer functional information regarding respiratory control, and feels that Gray's statement that all preceding theories "have consistently met with failure" is not supported by his analysis.

Gernandt (168) found that chemoceptor elimination leaving baroreceptors intact caused a reduction in the ventilation of about 36 per cent, agreeing perfectly with the previous observations of Bernthal and Weeks. It is concluded that the chemoceptors are responsible for a tonic reflexogenic respiratory stimulation. Of this 36 per cent, 13 per cent was found to be due to the aortic body, the remaining 23 per cent to the carotid bodies. After chemoceptor elimination, carotid compression was without effect upon respiration, nor did ventilation change as a result of destruction of the baroreceptors in an animal with chemoceptors previously destroyed. It is concluded that in this animal (cat) the baroreceptors are without direct effect upon respiration. Heymans & Pannier (169) found that increase of pressure in the chemoceptively denervated carotid sinus caused reflex respiratory inhibition, while decrease of pressure caused hyperpnea. No explanation of the diametrically opposed findings from these two laboratories is immediately apparent. Dripps & Comroe (170), from a study of the effects in man of breathing various percentages of oxygen (8 to 100 per cent), conclude that the chemoceptors are not tonically active.

Heymans, Bouckaert & Pannier (171) found that physostigmine augmented the excitability of the carotid body to acetylcholine, but not to other chemical stimulants, from which they conclude that acetylcholine is not involved as a chemical intermediary in the excitation of the chemoceptors, a conclusion further supported by the observations of Heymans & Pannier (172) that the respiratory effects of pressure changes in the carotid sinus were not affected by DFP. Van Damme (173) found that in spite of considerable anatomical differences the carotid bifurcation of the sheep possesses the same functional value as in other animals. Askey (174) reports seven instances of hemiplegia occurring immediately after carotid sinus stimulation in elderly patients with arteriosclerosis. Dickinson & Traver (175) reviewed the literature on carotid body tumors, presenting two cases of their own.

Walker, Smolik & Gilson (176) describe the effects of intra-

cisternal injection of potassium phosphate, interpreting them as results of medullary stimulation. Bucher (177) describes effects of intravenous morphine in rabbits, pointing out their similarity to effects of section through the caudal third of the pons. Walsh & Whitteridge (178), recording the activity of vagal fibers in cats and rabbits during the injections of suspensions of starch, obtained support for the view that vascular changes in the pulmonary circulation may account for the tachypnea seen with multiple pulmonary emboli. Oberholzer (179) found that some hypnotics promoted the expiratory, and others the inspiratory, components of the vagal respiratory reflexes. The same worker (180) studied the relationship between the circulatory and respiratory effects of afferent vagal stimulation.

Rall, Gilbert & Trump (181) found that the broncho-constriction resulting from pharyngeal stimulation is mediated not entirely by the vagus, but probably involves also some reduction in sympathetic tone. Bucher (182) described in the rabbit pulse-synchronous respiration apparently resulting from rhythmic changes in intrathoracic pressure associated with cardiac activity. Fleisch *et al.* (183) describe an expiratory effect of stimulation of the central end of the phrenic, apparently from pain fibers. Kalabuchov (184), studying the effect of temperature variation on respiration of various marsupials and true mammals, calls attention to the fact that the respiratory responses to temperature changes are frequently very different in closely related species.

#### THE LUNGS AND UPPER RESPIRATORY TRACT

Gagge, Allen & Marbarger (185) have described the "pressure breathing" equipment used in the A.A.F., and discussed its advantages. Allen & Gagge (186) found that anesthetized men breathed against pressures as high as eight inches of water without difficulty. Carr & Essex (187) made a thorough study of the effects of breathing against positive pressures upon the circulatory and respiratory systems of anesthetized dogs. They found that such pressures, continuous or intermittent, slow and deepen respiration, and that continuous pressure breathing may cause fatal apnea. Both types, if given over a period of three hours, may produce acute parenchymal and subpleural emphysema. Knoefel *et al.* (188) found in anesthetized dogs that 7 mm. Hg positive pressure applied continuously for three hours reduced cardiac output 41 per cent.

Drury and co-workers (189) described deleterious circulatory effects with resultant impairment of kidney function from 20 to 50 mm. Hg continuous pressure. Otis, Rahn & Fenn (190) present data on pulmonary relaxation and peripheral venous pressures, from which may be predicted the behavior of the mean venous pressure during pressure breathing. A method for estimating the distensibility of the lung is discussed. Rahn *et al.* (191) described the construction of a pressure-volume diagram of the thorax and lung. They found that with pressures up to 5 mm. Hg the work of breathing decreases, but at higher pressures it may increase as much as tenfold. It is obvious that the potentially harmful effects of positive pressures must constantly be kept in mind by those responsible for the design and tactical employment of oxygen equipment in aircraft.

Experiments of Wilson, Hall & Swann (194) did not support the suggestion that the development of negative mask pressures might account for some cases of loss of consciousness during free fall parachute descent.

Gray & Green (198) applied the method of Cournand *et al.* for measuring ventilation capacity to eighty-nine healthy aviation cadets. It is suggested that this procedure may be clinically useful as a ventilatory function test of the respiratory system. Macklin (199) showed by x-ray that in rabbits the pulmonary arteries are shortened and narrowed in passing from expiration to collapse. It is assumed that similar directional changes occur in normal breathing and that such an action will aid the heart. Moritz & Weisiger (200) concluded from their experiments on dogs that significant injury to the air passages of man was not likely to result from the breathing of air at any degree of coldness likely to be encountered in nonexperimental conditions.

Riley & Lilienthal (204) describe a method for the indirect determination of partial pressures in alveolar air which they believe gives more accurate values than can be obtained by standard methods of direct sampling. Müller (210) maintains that present knowledge of the dimensions and physico-chemical properties of the lung tissue is insufficient to justify application of diffusion laws and feels that it has not been proved that gaseous exchange in the lung is due to simple diffusion.

Boothby & Helmholtz (211) made roentgen kymographic studies of cardiac and respiratory movements. The contractions of



the diaphragm appear not to be simultaneous in different parts, but to have a sinuous character. The change in axis of the heart and the lowering of the diaphragm with positive pressure breathing are clearly shown. Starr & Friedland (212) found that placing the subject on the ballistocardiograph table in such a position that the axis of the heart in expiration was in the line of recording failed to reverse the normal respiratory variation. This variation, however, was reversed by positive artificial ventilation leading to the conclusion that it was due to changes in cardiac filling, and not to changes in position of the heart. Otis *et al.* (213) made a ballistocardiographic study of the effect of variations in intrapulmonary pressure or alveolar carbon dioxide tensions, and of pure oxygen inhalation. Cruickshank (214) found in the isolated heart-lung-head preparation in the rat that changes in frequency and amplitude of positive ventilation of the lung had no effect on cardiac output, but with negative ventilation an increase in tidal air volume resulted in an increase in cardiac output. Brookhart & Boyd (215) discuss circulatory effects of local variations in intrathoracic pressure, presenting results which appear to be of considerable significance in experimental measurements of cardiac filling and output. Houston (217) had resting subjects breathing 10.5 per cent oxygen voluntarily adjust respiratory volume at various levels. He found that doubling the resting ventilation increased the arterial oxygen saturation by 20 to 30 per cent, and suggests that clinical anoxia tests should be based on studies of arterial saturation rather than on the oxygen in the inspired air. Sartori (218) found in dogs that if the bronchi of one lung were tied the oxygen saturation of the arterial blood fell to half the original value, while tying off the whole pedicle was without effect. Scherf (219) discusses cardiac reflexes originating in the respiratory tract. He described one case in which arrest of breathing in any phase was always accompanied by immediate temporary cardiac standstill.

Work upon the following related topics has been published: effects of counter pressures to inspiration on circulation and respiration (192, 193); vital capacities of whites, Indians, and mestees (195); vital capacity in white females compared with Indian and other Oriental females (196); vital capacity of Bengalese youths (197); methods for measurement of water and heat loss from the respiratory tract of man (201, 202); the interpretation of water loss from the lungs (203); the relationships of alveolar and arterial oxy-

gen tension (205); the relation between alveolar and cutaneous respiration in frogs (206); lung nitrogen elimination and effectiveness of pulmonary gas mixing (207); the physiological factors governing gas exchange (208, 209); blood oxygen as affected by intratracheal pressures and anesthetic mixtures (216); respiratory efficiency at altitudes (220); the respiratory function of the larynx (221); prediction value of breath holding tests (222); respiratory data on monkeys (223); and respiration in turtles (224).

#### RESUSCITATION

The most significant study dealing with the subject of resuscitation in recent years is that of Negovski. Originally published as a monograph, extensive excerpts have appeared during the past year in the *American Review of Soviet Medicine* (225 to 231). This investigation has been characterized equally by its thoroughness in execution and sound conservatism in interpretation. From studies upon several hundred animals, mainly dogs, it was concluded that arterial infusion of blood, plus epinephrine and glucose, together with powerful artificial respiration, constituted the most effective method of revival. The heart action usually ceased shortly before, or more rarely with, the last breath. Revival was possible only in those cases where the duration of clinical death did not exceed six to eight minutes, and the chances of revival were practically nil after five minutes. Severe pathological changes of the central nervous system were obvious after clinical death of more than six minutes' duration, these observations emphasizing the vital importance of speed in the initiation of resuscitative procedures.

Jacobi *et al.* (232) reported success in the treatment by intravenous oxygen administration of twelve dogs suffering from experimental toxic shock, and three human beings with secondary traumatic shock. Grodins, Lein & Adler (233, 234), from studies of the acid-base balance of the blood of dogs during asphyxia and resuscitation, conclude that there is no deficiency in the chemical stimuli to the respiratory center during the entire course of obstructive asphyxia, or in the latter stages of nitrogen asphyxia, and question the use of carbon dioxide in resuscitation. Wulff (235) found that resuscitation was more difficult after asphyxiation with pure carbon dioxide than after carbon dioxide-oxygen mixtures or pure nitrogen. Schwerma, Ivy *et al.* (236) made a study in dogs of various methods of resuscitation from asphyxia by illuminating gas

and by pure carbon monoxide. While accurate comparisons are difficult, the results seem to indicate a very questionable advantage of carbon dioxide-oxygen mixtures over pure oxygen. Vining and co-workers (237) found 7 per cent carbon dioxide in oxygen much more effective than pure oxygen or air in the treatment of goats and dogs acutely asphyxiated with carbon monoxide.

Comroe & Dripps (238) from studies on three human patients, two of them with complete respiratory arrest, were impressed by the ineffectiveness of the Schafer as compared with the Eve method, and with intratracheal oxygen insufflation. They emphasize the need for a comprehensive study of the relative effectiveness of various resuscitative methods. Ross (239) reports an analysis of 3,352 cases in which artificial respiration was administered during the years 1940 to 1944. No injuries from resuscitative procedures were reported. The data do not permit an evaluation of the relative efficacy of various methods. Schwerma & Ivy (240) anaesthetized and curarized twenty normal dogs and administered artificial respiration for one to three hours by means of the E. & J. Resuscitator. Autopsy three days later revealed no evidence of pulmonary damage as a result of the artificial respiration. In five anaesthetized dogs given artificial respiration with the E. & J. Resuscitator in the presence of normal breathing, there was no evidence of emphysema. In the experiments of Schwerma, Ivy *et al.* referred to above (236), mechanical resuscitation appeared to have little or no advantage over manual methods in the treatment of dogs asphyxiated by carbon monoxide. A device known as a "pneumatic balance resuscitator" has been developed by Burns (241) and used clinically by Olson (242).

During the past year there has appeared a book by Drinker entitled "Pulmonary Edema and Inflammation" (243), welcome equally to the physiologist and clinician. To the four lectures upon which the book originally was based, there has been added a fifth chapter on artificial respiration in which the author outlines the requirements which must be met by any satisfactory method of artificial respiration, and very convincingly presents the case against the mechanical resuscitator. His observations upon the importance of the Hering-Breuer reflex in resuscitation will be of particular interest to Gesell who, almost alone among physiologists, has insisted upon the excitatory function of the afferent vagus.

The apparent tendency toward increasing use of mechanical

resuscitators appears to the reviewer to be extremely ill-advised. The one fact regarding resuscitation which must be emphasized above all others is the importance of speed. The fact that five minutes after breathing has stopped the chances of revival have become almost negligible means that in the great majority of cases artificial respiration must be initiated by the man on the spot with equipment immediately available, viz., his two hands. The procedure of choice would appear to involve the initial use of the Schafer method, replacing it with the Eve method if and when it can be done without interruption.

#### METHODS AND EQUIPMENT

Bloomfield (244) presents a method for the optical recording of intrapleural pressure simultaneously with right heart pressure changes, utilizing the intracardiac catheterization technique in conjunction with a modified Hamilton manometer and pleural catheter. Kety & Schmidt (245) describe a method for the quantitative determination of cerebral blood flow in man based on arterial and internal jugular concentrations of an inert gas. Lilly (246) describes studies on the mixing of gases within the respiratory system utilizing a new type of nitrogen meter. Millikan (247) studied the speed of response of arterial oxygen saturation to rapid changes in equivalent altitude using instruments whose time resolving power is a small fraction of one respiratory cycle. Rahn *et al.* (248) describe an apparatus for the automatic sampling and continuous analysis of alveolar air during normal respiration.

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DEPARTMENT OF PHYSIOLOGY  
UNIVERSITY OF MICHIGAN  
ANN ARBOR, MICHIGAN

## NERVE AND SYNAPTIC CONDUCTION

BY FRÉDÉRIC BREMER

*Laboratoire de Pathologie Générale, Université de Bruxelles, Brussels, Belgium*

The review deals primarily with papers on nerve and synaptic transmission published from September 15, 1945 to September 15, 1946, but relevant material has been drawn from earlier reports, and especially from papers published in Europe during the war. The reviewer regrets that omissions may have been made in the present account, since the delivery of foreign periodicals in Belgium is still irregular and delayed.

### NERVE CONDUCTION

*Chemical correlates.*—There is a general agreement on the proposition that nerve conduction is an electrochemical event, in which metabolic processes, intimately connected with the production of electrical potentials and currents, are essential agencies. The numerous data already collected, however, cannot yet be grouped in a coherent theory, and one is still far from a thermodynamic formulation of the process of nerve conduction. Opportunity for a stimulating exchange of views on the various aspects of the problem was provided at the conference instigated by the New York Academy of Sciences and held in February, 1946. The discussion has been mainly focused on the question of the functional significance of the metabolism of acetylcholine in nerve tissue. The greatest difficulty facing the conception, which Nachmansohn and his associates have supported by various and impressive arguments (1), is that a "chemical wave transmission" [Hill (2)], with similar properties, is observed in all excitable tissues whose elements are sufficiently elongated to allow the observation of an impulse propagation, and that among such biological objects many, like the unicellular algae, the skeletal muscle fibers, and the sensory and adrenergic fibers of vertebrate nerves, show no evidence of an acetylcholine metabolism. One hesitates to admit that a fundamental biological phenomenon, with such general homologies of properties, should depend for its mechanism upon essentially different chemical processes. Yet, comparative biochemistry offers instances of the substitution of different substances for homologous functions, sometimes in zoologically related orders, so that the objection is not a decisive one.

Whatever may be the fate of the generalization of the acetylcholine theory, in definite cases new observations have been brought, which are consistent with the conception that the release and rapid removal of acetylcholine is an essential event during the conduction of the nerve impulse. A reversible abolition of the action potential of the giant axon, of the fin nerve of the squid, and of the abdominal chain of the lobster under the action of physostigmine and of the newly-discovered powerful anticholinesterase, diisopropyl fluorophosphate (DFP) has been described (3). The degree of reversibility of the effect after washing in sea water was found to be parallel to the amount of cholinesterase reappearing, and the lack of effect of prostigmine could be attributed to its absence of penetration through the lipoid membrane surrounding the axon. The opposite conclusion, which had been derived by Gilman and his associates (4) from similar experiments on the nerve of the bullfrog, was attributed to the physicochemical particulars of the frog nerve, resulting in a great difficulty in the exact assay of their enzyme content. However, physostigmine, in the researches of von Muralt and his associates (5) on the frog nerve, has never resulted in an appreciable accumulation of acetylcholine. This negative result contrasted with the accumulation produced in the stimulated frog nerve when it had been intoxicated by monoiodoacetic acid, an effect which was attributed to an inhibitory action of the drug on the resynthesis of the liberated acetylcholine into its hypothetical precursor, pro-acetylcholine (5, 6). The two other active substances whose liberation has been demonstrated during the process of nerve impulse transmission in the amphibian and mammalian mixed nerves are thiamin and as yet unidentified organic substance detected polarographically, designated substance A/4 (5). The meaning of their relation with nerve conduction is still a matter of conjecture. It has been surmised that, like acetylcholine itself, they are involved in the chain of restitution processes underlying the functional restoration (the repolarization) of the nerve fiber. The hypothesis has been developed by von Muralt in a recent monograph (5), where he has summed up the contributions of his laboratory. This conception differs thus from the theory of Nachmansohn (1), which implies that the restoration of the nerve fiber after conduction consists essentially in the enzymatic resynthesis of acetylcholine from its breakdown products, and which sees in the energy-rich phosphate bonds, especially phosphocreatine, the



main source of energy. Measurements, performed on the electrical organ of the electric eel, have led to the conclusion that the energy released by phosphocreatine breakdown and lactic acid formation during the discharge of the organ (discharge assumed to be homologous to a compound nerve action potential) is adequate to account for the electrical energy released by the same discharge (1, 7).

The enzyme system which synthesizes acetylcholine in nervous tissue has been found by Feldberg & Mann (8) to be more complex than it was initially supposed. They have also measured the ability of the various parts of the nervous system to synthesize acetylcholine and confirmed the noncholinergic nature of the afferent nerves (dorsal spinal roots and also optic nerve).

The hypothesis that the conduction mechanism could depend in different nerves on different active substances gains some ground when one considers the recent results obtained by von Euler (9). The pharmacological study of direct extracts of sympathetic nerves (cattle and horse) has shown him that the active substance they contain differs from epinephrine but is biologically and fluoroscopically identical with noradrenalin and with the sympathin of Cannon & Bacq (10). The substance can be extracted and is probably liberated physiologically from the whole length of the adrenergic nerve fiber. It is also present, in substantial amounts, in sensory nerves to the skin and to a lesser degree in dorsal and ventral spinal roots.

The chemical aspects of wallerian degeneration of nerve fibers has continued to arouse the interest of the physiologists hoping to find in these studies new evidence of the functional significance of the active substances. The degeneration of the myelinated fiber (guinea pig sciatic) is characterized by a rapid and progressive fall of its content in the following substances: acetylcholine (11, 5); cholinesterase (but not pseudocholinesterase), a change contrasting with the marked increase of the content of the neuroma (12, 13); free and bound thiamin (14); and phosphatases (15). These latter enzymes, as well as the respiratory enzymes (1), appear to be located only in the axis cylinder, while cholinesterase, and possibly also thiamin, are concentrated at the neuronal surface or the adjacent myelin sheath (1, 5).

Nerve regeneration seems to be also controlled by active substances. From brain and other tissue extracts, a substance has been isolated which exerts a powerful stimulating action on nerve re-

generation (5, 16). This N-R growth substance, as it has been called by von Muralt, is not a protein and is species-unspecific. Its effects which are particularly striking when the cornea test is used are accompanied by a parallel intensification of the increase of vascularization of the tissue, which normally accompanies the growth of regenerating axons (17).

*Electrical correlates.*—The electrophysiological description of nerve conduction has still to account for the fact, disturbing for the classical membrane theory, that, in some nerves at least, the voltage of the action potential exceeds notably the voltage of the resting potential measured at the same time. Hodgkin & Huxley (19) who discovered this "overshoot" in the giant axon of the squid—an observation which, as is known, was soon confirmed by Curtis & Cole (18)—have made a new study of the phenomenon and have demonstrated its existence on the crustacean nerve fiber. The conclusion of their researches is that the overshoot is too large to be explained by a liquid junction between the axoplasm and the microelectrode and that it must be due to a genuine reversal of potential at the surface membrane of an active section of the nerve. Among the various suggestions discussed by them, the hypothesis of Curtis & Cole (18), based on the two demonstrated facts of the existence of an induction component in the axial impedance of the squid axon and of the dropping of the nerve membrane resistance to a finite small value during activity, has the advantage of explaining the positive phase which is a characteristic feature of the action potential of the squid axon, recorded with an internal electrode. In a recent reformulation of the membrane theory, Höber (20) has put forward the hypothesis of the liberation of nonpolar-polar organic ions in the active nerve producing a reversed resting potential when in very weak concentration. Another explanation, mentioned by von Muralt (5), could possibly be drawn from the catelectrotonus experiments of Tasaki & Takeuchi (21) on single myelinated fibers, suggesting that the measured resting potential represented only a fraction (about half in their experiments) of the e.m.f. (ionic polarization) of the resting membrane.

A theory of nerve impulse transmission in peripheral myelinated fibers, based mainly on the work of the Japanese physiologists on single fibers of the frog sciatic and on his own confirmatory experiments, supplemented by histological and microstructural ob-

servations, has been presented by von Muralt (5). The author stresses particularly the importance of the transverse membrane at the Ranvier node, which he has discovered, for the mechanism of the saltatory propagation of the nerve impulse in myelinated fibers.

The accommodation phenomenon, the physiological and pathological importance of which is becoming increasingly evident, has been studied on frog, cat, and human nerves. A parallelism between accommodation and electrotonus has been disclosed by the comparison of motor and sensory fibers, and by the similar influence of variations in the calcium content of the bathing fluid on the electrotonic changes of excitability and on the state of accommodation of the nerve fiber (22). In man (23), ischemia of the nerve trunk and calcium deficiency have been found to have similar and synergic effects on nerve accommodation. The typical symptoms of tetany (paresthesias, spasms, and the Trousseau phenomenon) could be connected with spontaneous activity of the proximal region of the longest fibers of the arm nerves.

The excitatory properties of the artificial synapse (ephapse) formed by severed mammalian nerve have been further analyzed by Skoglund (24). A conditioning volley in the "prefibers" is followed by periodic variations in excitability in the "post fibers" (supernormality followed by subnormality), even when the afferent volley is subliminal for the artificial synapse. The phenomenon is characteristically modified by electrical polarization of the ephapse. Increase of stimulus frequency causes in it a cumulative depression, the amount of which depends upon the accommodation properties of the "postfibers" and upon the duration of the nervous impulse in the "prefibers."

Nerve degeneration has been studied oscillographically by the ingenious method of sectioning one of the two roots of the phrenic nerve (cat), the intact fibers of the nerve issued from the other root serving as control (25). In conformity with histological data on the degeneration of the axon the conclusion has been reached that failure of conduction is abrupt at randomly scattered foci. This fact does not contradict the observation of a gradient of functional depression (fatigability) in the degenerating nerve, having a possible relation with the centrifugal alteration of the myelin [(26), see also (5)]. A similar centrifugal gradient of depression has been observed in the asphyxiated nerve of the cat (27).

Nerve regeneration provides an opportunity for comparing in the same animal the conduction rates of normal medullated fibers with those of fibers (in the proximal and distal stumps) in which the relations between sheath thickness, axon diameter, and internodal distance have been experimentally altered. Such measurements have been made upon rabbit mixed nerves which had been allowed to regenerate for different times after interruption by crushing (28). A direct dependence of conduction velocity upon myelin sheath thickness has been found. The meaning of the correlation is not yet clear. Other dimensions and qualities of the myelinated fiber, i.e., the chemical composition of its sheath, may also play a part in controlling its velocity. No relation was found in regenerating fibers between conduction velocity and internodal distance.

An analysis of the process of anoxia of the nerve fiber has revealed the interesting fact that, on the frog A fibers, the average time to suppress activity is significantly smaller when both the stimulated and the conducting regions are anoxiated than when only the leading-off section of the chamber is deprived of oxygen, as if the launching of the impulse were more easily suppressed than the blocking of its conduction (29).

A study has been made of the functional modifications of the frog nerve immersed in a solution of glucose buffered by bicarbonate (30). One observes a progressive increase of the rheobase (with little change of the chronaxie) and a progressive decrease of the voltage of the spike (after an initial increase) and of the velocity of conduction. The parallelism of the curves expressing the evolution of these functional alterations with the curve of nerve resistance suggests a causal relation.

The phenomenon of thermic narcosis of nerve fibers shows characteristics which have a bearing upon the theories of conduction. It has been studied comparatively (31) on the nerves of the two European frogs, *Rana temporaria* and *Rana esculenta*, in which the temperatures of paralysis differed by about 8°C. The nerve of *Rana temporaria* lost its excitability and conductivity at the remarkably low temperature of about 32°C., which was still further lowered by potassium and by anesthetics. The different fibers of the A group are differentially heat-sensitive, the  $\gamma$  ones being the more resistant (cf. 30). The reversibility of the phenomenon is rapid and complete. The voltage curves of the action potential

(spike and negative after potential) as a function of temperature show no parallelism with the curve of the resting potential. Thermic narcosis coincides in the two species with the same fall of resting potential of 8 to 9 mv. The immediate cause of the loss of conduction, which is preceded by a slowing of its velocity, is presumably the rise of threshold of the fibers. At a time when conduction is abolished, a local cathodic response is still observed. The initial perturbation leading ultimately to paralysis is probably the thermic inactivation of the nerve enzyme mechanisms (according to experiments on the respiration of brain tissue of the two species of frog).

#### NEUROMUSCULAR CONDUCTION

The electrical and pharmacological aspects of neuromuscular transmission have been studied on the isolated sartorius of the frog by Coppée (32). The conclusions of this extensive work, published in Belgium during the war, are in the main in agreement with the results and interpretations of Eccles, Katz & Kuffler (33). There are however points of divergence. From observations on curarizing agents, not hitherto studied electrophysiologically, the author has come to the conclusion that the endplate potential is a complex phenomenon. He regards it as the action potential of what he calls the *sole motrice*, an organite intermediate between the nerve terminals and the muscle fiber, and he interprets the slow declining phase of the potential (which is often preceded in its tracings by a brief spike) as a negative after-potential. The paper reports numerous pharmacological and oscillographic observations. Its general conclusions are that curarization, decurarization, facilitation, and potentiation can all be explained by the consideration of these factors: excitability of the *sole motrice*, voltage of the endplate potential, and excitability of the muscle fiber; as well as the fact that the role of acetylcholine in neuromuscular transmission is only a subsidiary one.

Katz & Kuffler have continued their work on neuromuscular mechanisms by a study of the response of crustacean muscle to motor nerve impulses. They found (34) that the junctional potentials were similar to those observed in vertebrate muscle. An important difference, however, is that, depending upon the number and frequency of nerve impulses, local or propagated muscle responses are set up. They also examined the effects of inhibitory nerve impulses on the junctional potential changes and the subse-

quent muscle responses (35). While the inhibitory impulses do not evoke any electrical responses of the muscle fibers, they reduce the junctional potential in proportion to the time interval separating them from the subsequent motor impulses. This reduction of the junctional potential is accompanied by the prevention of the propagated muscle impulse which accompanies it normally and by a depression of the local contraction at the junctional region. The contractile process is also depressed by a direct action, the mechanism of which is still obscure [cf. Marmont & Wiersma (36)]. Curiously enough, the inhibitory impulses do not affect the facilitatory process responsible for the progressive increase of the successive junctional potentials evoked by a repetitive stimulation of the motor nerve.

From an electromyographic study of the small ventral root fibers innervating skeletal vertebrate muscles (frog and cat), it has been concluded (37) that these fibers, which have no motor (contractile) effect, exert a sensitizing action on muscle receptors, presumably the muscle spindles.

The possibility of a reinnervation of denervated muscle fibers by the branching of adjacent regenerating fibers has been confirmed by new researches on cat muscles (38) and on poliomyelitic patients (39), with favorable conclusions concerning the therapeutic value of procedures aiming at the creation of such reinnervation on partially denervated muscles.

#### CENTRAL TRANSMISSION

*Synaptic potentials.*—The study of synaptic conduction is actually centered on the analysis of the properties and the functional significance of the prolonged negative potentials of the spinal cord, which are evoked by reflex stimuli, and which diffuse electrotonically along ventral and dorsal roots, as discovered by Barron & Matthews (40). Two important papers by Eccles (41) and Eccles & Malcolm (42) have been devoted to these potentials, which had also been studied by Bremer, Bonnet *et al.* (43, 44) in their researches published during the war on central summation and inhibition. There is general agreement on the great physiological importance of the phenomenon, whose properties, whatever may be the exact mechanism of its production, certainly justify the epithet of "synaptic," applied by Eccles to the ventral root potentials. The two last groups of authors agree in locating the site of the pro-

duction of the potentials at the synaptic surfaces of the nerve cells (motoneurons and interneurons), of which they would be a local and graduated electrical response to the impinging nerves impulses, homologous to the similar negative potential at the sympathetic ganglion synapses (45) and to the endplate (junctional) potential at the neuromuscular junction of skeletal muscle fibers (32 to 35). They would all perhaps represent a specialized modification of the local electrical response of the nerve and muscle fibers which is evoked by subliminal stimuli (see 46) and which may be exaggerated in pathological condition of the excitable tissue. As curarin and similar agents do for the endplate and also for the ganglionic potential (45), depressing agents or conditions (anesthetics, or postreactional refractoriness) by blocking synaptic transmission allow a recording of the potentials undistorted by the superimposed spikes of the cellulifugal impulses. The same agents reduce or suppress the repetitive excitation of motoneurons by the after-discharge of interneurons, a complication which can also be eliminated by the use of a monosynaptic path. These methods [(41), (cf. also 40)] have clearly shown that the setting of postsynaptic impulses is determined by the rate of rise, the absolute value, and the duration of the ventral root negative potential acting apparently as a catelectrotonic stimulus for the motoneuron, and itself evoked at the synaptic surfaces of the nerve cell by the action currents of the presynaptic impulses. No definite evidence has been found of an action of physostigmine or prostigmine on synaptic potentials, a negative result which contrasts with their increase by strychnine and by curarine (41).

Since the action potential of the nerve cell has properties differentiating it from the action potential of the axon [(47, 48); but see (49) for the bipolar dorsal root ganglion cells of selachian fishes], the complexity of the electrical processes involved successively by synaptic transmission, in the simplest central path, is already obvious. These complications have been revealed by the analysis of the mechanisms of central summation and inhibition (see below).

An explanation of the production of synaptic potentials has been suggested (50) as a part of an electrical hypothesis of synaptic and neuromuscular transmission. The theory is based on geometrical and electrophysiological considerations and on Hill's local potential principle. It assigns a subsidiary role to acetylcholine as a mediator in the transmission mechanism at synapses of sympa-



thetic ganglions and possibly also vertebrate muscle fibers.

Differences of experimental conditions and technics, and the very nature of the synaptic potentials, explain the divergences of description and of interpretation of the ventral and dorsal potentials by the different authors who have studied them. The dorsal root potentials offer a special difficulty for analysis, on account of the fact that, if their site of production is, as it seems plausible (43, 44, 42), the synaptic membrane of interneurons, their electrotonic transmission along the root fibers must be trans-synaptic. The discharge of impulses along these same fibers (dorsal root reflex), which is often clearly associated with the slow negative potential, has its simplest explanation in the hypothesis that these antidromic impulses are fired off by a cathodal polarization of the central terminals of the fibers (42). On the other hand, the quality of the synaptic potentials of being protoplasmic reactions explains that they are followed by a period of postreactional subnormality of long duration (cf. 46). This subnormality is especially obvious in the case of dorsal root potentials, probably because it is not statistically compensated by a recruiting process. Its time course has been found to parallel the evolution in the spinal cord of the inhibitory effect of a conditioning volley of impulses (44).

The neuron structure of the vertebrate retina confers on the facts disclosed by its study a general significance for central nervous physiology. Here, as in the spinal gray matter, slow potentials appear to be the electrical intermediary in the processes of neuron excitation and inhibition which follow illumination or sudden darkness (see 51). New evidence of this mechanism has been brought by Granit's experiments of polarization of the eyeball of the cat and recording of single fiber discharges from the optic nerve (52). The electrical stimulus, like the physiological one reveals, in the retina, structures having apparently some inherent differential polarity. These properties throw new light on the general significance of pre- and postexcitatory inhibition in the retina and suggest the presence in it of reciprocal innervation.

*Summation and facilitation.*—Experiments on the frog spinal cord have demonstrated again the multiplicity of processes underlying the central summation of two centripetal volleys, and allowed their analysis (43). The summation curve (strength of the reflex twitch as a function of the interval of the two volleys) can be dissociated experimentally in its components. The three different

mechanisms whose intervention had been postulated, sometimes to the exclusion of each other, can be shown to be successively at play in central summation (*addition latente*): (a) a very brief excitatory action requiring the convergence of the centripetal impulses by different paths for its detection; (b) a subsequent slow phase, of exponential decay apparently homologous with the summation process of two subliminal volleys of motor impulses at the curarized neuromyal junction; (c) a third, still longer phase, of variable duration, which, by its parallelism with reflex after-discharge and by its similar elective sensibility to the depressing action of barbitalurates, represents probably the delayed re-excitation process of the motoneurons by impulses issued from the internuncial system of the cord (cf. 53).

The slow process supposedly homologous with the latent modification responsible for the summation at the neuromuscular junction is the only one in action, when the two centripetal volleys are transmitted by the same afferent fibers to a barbitalized center. The similarity of time course and shape of its representative curve with the trace of the synaptic (ventral root) potential, and the direct evidence that, in the deeply narcotized spinal cord, the summation of two such successive negative slow potentials leads to the emission of an axonic impulse when a critical degree of depolarization is reached (41), certainly justify the simplifying hypothesis that the two phenomena are expressions of one and the same synaptic process, and that facilitation is due to the summation of the synaptic potentials (40, 41). However, this simplification still encounters difficulties (43) and the possibility cannot yet be excluded, in the reviewer's opinion, that, as it has been shown for neuromuscular junction, especially the crustacean one (34, 35), a summation mechanism does exist in the spinal cord which is independent of the synaptic potentials, whereby these last ones are themselves facilitated.

Rosenblueth *et al.* (54), studying spinal reflexes of the cat, have described delayed and prolonged postreactional facilitation, with effects quantitatively different for the ipsilateral and crossed reflexes of the same muscle (the quadriceps) and for the several components of each reflex. This prolonged facilitation of motoneurons is related by them to a spinal mechanism capable of self-sustained activity, not explainable by reverberating circuits in the gray matter.

*Acetylcholine as a synaptic transmitter.*—The theory of acetylcholine as a synaptic transmitter has been discussed by Feldberg (55), who, in concluding a review of all the experimental data, expresses the opinion that the present position of this theory is anything but settled. The latest pharmacological contributions to the solution of the problem are rather deceiving. The study of the recently discovered anticholinesterases of powerful and long lasting action, has revealed puzzling discrepancies (56). The most surprising results are those of Heymans and his co-workers (57, 58). They failed to observe any effect of DFP (as also of prostigmine) on the central apparatus of the carotid sinus reflexes and of the respiratory center. But they also found that DFP, at the same doses which almost totally inhibited the cholinesterases of the blood plasma and red corpuscles, while sensitizing the heart to acetylcholine, failed completely to sensitize it to the vagus, and had no effect on the arterial blood pressure, heart rate, respiration, salivary secretion, intestinal peristalsis, and size of the pupils. A subsequent injection of physostigmine, or prostigmine had its usual cholinergic effects, even exaggerated. The very completeness of these negative results weakens perhaps rather than strengthens their bearing on the problem of acetylcholine as a central transmitter, and suggests an explanation based either on permeability factors controlling the access of the drug to the synaptic regions or on a possible effect of it on the synthesis of acetylcholine. In consideration of the last eventuality, the absence of cholinergic effects of DFP would be an objection to the theory of the chemical mediation of nervous impulses, and a most serious one, if it were demonstrated that the same dose of the drug resulted in a distinct increase in the output of acetylcholine during the stimulation of a cholinergic nerve in a perfusion experiment.

There remain the contradictory results obtained with physostigmine and prostigmine on different reflex preparations, e.g., the knee-jerk and the spinal flexor reflex (see 55). A possible explanation of these discrepancies might be that acetylcholine, at the very small concentrations resulting from its leaking at the synaptic regions, would only affect neurons (i.e. the internuncial aggregates) having a special excitability (weak accommodation) or a tendency to autorhythmic discharges. Its role in central transmission would then be limited to the causation of reflex after-discharge (59).

However a serious objection to any theory assigning a role to

acetylcholine in central transmission is the fact that sensory fibers of the vertebrates are not cholinergic (see 55). A new indication of this fact is suggested by the nerve suture experiments of de Castro (60). He succeeded in substituting functionally the central afferent fibers issued from a sensory ganglion (the ganglion nodosum of the vagus) for the preganglionic fibers of the superior cervical ganglion. But he points out that physostigmine did not potentiate the discharge of such reinnervated ganglions, while it did so for the discharges of normal ganglions and of ganglions the preganglionic fibers of which had been substituted by cholinergic fibers, like those of the efferent vagus or hypoglossus. In the "afferent vagus" sympathetic ganglion, synaptic transmission would be assured by a purely electrical mechanism, while in the "efferent vagus" and "hypoglossus" ganglions it would imply a dual, electrohumoral mechanism, as in the normal one.

*After-discharge.*—The properties of reflex after-discharge have been studied on the spinal preparation of the frog and the toad, and its mechanism discussed (59). The authors have taken advantage of the fact that the reflex after-discharge of the so-called "tonic" muscles of the batracians (which are probably the homologous of the red muscles of the mammals) is characterized by its intensity and its very long duration. No proprioceptive or interneuronic reverberation could apparently explain such durations, for the phenomenon is exaggerated both by the complete deafferentation of the active limb (in the contralateral reflex of the toad) and by a transection of the cord leaving for the reflex only its terminal lumbosacral segments.

After-discharge was found to be electively sensitive to electrotonic and pharmacological agents. The same differential sensitivity was found for the primary wave and the subsequent after-discharge of the response of the acoustic cortex of the cat to a centripetal volley. In both cases particularly striking was the reinforcement of after-discharge by very minute doses of acetylcholine (intra-arterial injection in the unanesthetized animal), and its abolition by barbiturates. These experiments have led to the conclusion, at which Rosenblueth and his associates (54) have also arrived, that an important, if not exclusive, mechanism of after-discharge resides in the tendency of neuron aggregates to self-sustained activity, or, what may be the same, to rhythmical fluctuations of threshold (cf. 24). This tendency might be strongest in the inter-

nuncial system and favored there by the leaking of a trace of acetylcholine in the synaptic region (59). Such mechanism does not of course exclude, as a contributory factor in the causation of after-discharge, the circuiting of impulses in chains of interneurons, which has been assumed by Lorente de N6 (53).

New arguments in favor of this conception of reflex after-discharge can be mentioned. Larrabee & Bronk (61) have confirmed their previous observation that an after-discharge of sympathetic ganglion cells, a case in which of course no interneurons can be postulated, may follow a strong preganglionic stimulation. And Renshaw (62) has made the important observation that a centripetal volley entering the spinal cord over alpha fibers of ventral (motor) roots in cats and rabbits evoke rhythmic action potentials in the ipsilateral ventral horn (microelectrodes) which persist, in progressively decreasing number, for 30 to 50 msec. The discharge represents the activity of neurons of the ventral horn (presumably large interneurons), and can be conditioned by antidromic or normodromic volleys. It may have a relation with the prolonged negative potential set up in the spinal gray matter by an antidromic volley and recorded in the dorsal root (42).

*Inhibition.*—In spite of the progresses made during the recent years, this important chapter of central nervous physiology is still obscured by difficulties and uncertainties. A fact which no doubt contributes to this confusion is that the term inhibition is rather loosely applied to categories of central depression which are not, or may not be, homologous. For instance, it often designates the postreactional depression of a reflex and the silent period of the electromyogram which follows the transmission of a tendon-jerk reflex. This generalization may prove ultimately to be justified by the demonstration of the fundamental identity of mechanism of these postreactional refractorinesses with the depression of central activity resulting from afferent volleys not evoking themselves any discharge of the same motoneurons, or even any visible discharge. But provisionally, and for the sake of clearness, the term inhibition should, in the reviewer's opinion, be reserved to central depressions which cannot be attributed to the postreactional refractoriness of motoneurons. Even with such limitations, there are still great incertitudes as to the phenomenal homogeneity of central inhibitions, such as the inhibitory component of reflexes obeying reciprocal innervation, the paleocerebellar inhibition (63), the cortical suppres-

sion and extinction phenomena, the widespread inhibition of reticular origin described by Magoun & Rhines (64), and the diffuse reflex inhibitions which characterizes animal hypnosis and simulated death of vertebrate and arthropods.

The discovery, by Renshaw and by Lloyd (65) of the direct inhibition of monosynaptic reflexes will certainly prove of great importance for the understanding of some of the inhibitory phenomenon listed above. Its exact mechanism is still uncertain. The responsiveness of the motoneurons, as tested by the size of their antidromically evoked action potential, is apparently not depressed by the inhibitory volley (66). From this unexpected fact, and from the oscillographic characteristics of the inhibited reflex, Renshaw has concluded that at least part of the interaction (presumably electrical) responsible for direct inhibition is between contiguous premotor (presynaptic) fibers, without direct depression of the motoneurons. The validity of this conclusion may perhaps be questioned. For, apart from a reluctance to admit that a powerful, and probably functionally very important, inhibition phenomenon should depend on the hazardous effect of a fiber interaction, the objection which comes to mind is that the direct inhibition of motoneurons, resulting in a rise of their threshold for normodromic impulses, might not affect the all-or-none response of the same motoneurons to an antidromic volley. On the other side, the striking parallelism of the time-curves of the excitatory process and of the corresponding inhibitory process (see 65) suggests some kind of electrotonic action or interaction [for the theoretical possibilities, see (67)], as the basis of direct inhibition, rather than a chemical mechanism which would not result in such a parallelism of the two central effects.

The functional significance of direct inhibition of monosynaptic reflexes is still a matter of conjecture. Lloyd (65) has made the interesting suggestion that it could be the mechanism of the reflex fractionation and limitation which characterizes myotatic reflexes, while the postreactional subnormality of interneurons would account for the inhibitory component of plurisynaptic reflexes, like the ipsilateral flexor and crossed extensor ones. But tendon jerks and tonic myotatic reflexes are inhibited by afferent impulses other than the muscle proprioceptive ones (see 67a) and their abolition is also a feature of the generalized inhibition evoked by stimulation of the bulbar reticular formation (64).

A powerful inhibitory phenomenon has been described on the spinal frog and submitted to an oscillographic and pharmacological analysis (44). A functional condition of the spinal center was chosen, such that the summation of two successive maximal sensory volleys was necessary to elicit a reflex discharge, while the delayed facilitatory process (due presumably to internuncial activity) was not in play. If then a conditioning stimulus, identical in strength to the two reflexomotor stimuli and applied on the same or on the opposite nerve, preceded by a sufficient interval the pair of testing stimuli, it exerted a powerful and long-lasting inhibition on the reflex twitch. Its duration could attain ten seconds. From all its properties (the cumulative effects of successive inhibitory volleys, the duration of the effect identical to the duration of the postreactional depression following a reflex twitch, and the reinforcement by cataleptotonus, by strychnine, and by yohimbine) the inhibition phenomenon betrayed its relation with the postreactional subnormality of nerve fibers and nerve cells described by Graham (68) and by Lorente de N6 & Graham (69). As in the experiments of Hughes & Gasser (70) and of McCouch *et al.* (71) on the spinal cord, the site of this postulated subnormality was located at the internuncial level. An indication of this site was given by the recording of the dorsal root electrotonic potentials evoked by the conditioning and testing volleys in combination. The depression of the dorsal root potentials evoked by the pair of testing volleys was found to parallel the intensity of the inhibitory effect, witnessed by the myogram of the reflex twitch simultaneously recorded.

#### SPONTANEOUS ACTIVITY OF NERVE CELLS

Characteristic features of reflex action admittedly require for their explanation the assumption of some kind of self-sustained activity in the nerve centers, outlasting considerably the duration of the elementary synaptic processes. A possible mechanism of this self-sustained activity could be, as suggested, by Lorente de N6 (53), the re-entry of nerve impulses travelling along reverberating circuits in the internuncial system of the gray matter. But the theory has been criticized (44, 54), and the alternative hypothesis of a true autorhythmicity of nerve centers, expressing itself, either as an apparently spontaneous activity, or as a repetitive discharge of neurons outlasting the centripetal volleys, has gained ground. The conception of spontaneity needs of course qualification, be-



cause the necessity of a minimal chemical, or nervous stimulation for the manifestation of neuronics automatism has been demonstrated by the study of the functional condition of completely, or almost completely, deafferented neuronics aggregates (see 72).

The spontaneous activity of the spinal gray matter has been analyzed in the curarized cat (73). The electrospinogram is normally characterized by very small and irregular waves, but synchronization is easily obtained, at least for the anterior horn neurons, by a strong reflex stimulation, especially in the after-discharge of the response, by barbiturates associated with a light dosis of strychnine, and in the initial stage of asphyxia. The electrospinogram has then great similarities with the electrocorticogram of the barbitalized animal, and its "spindles" may correspond in time with those of the motor cortex. Complete synchronization of the anterior horn neurons is observed in the strychnine tetanus (74). Its waves, of high voltage and of a surprising regularity, are in phase along the whole length of the spinal cord. Their frequency, which varies from about 18 cycles per sec. initially to 10 cycles per sec. at the end of the tetanus, is accelerated by nicotine and by a catelectrotonus, slowed (eventually to a complete reversible standstill) by a puff of ether and by an anelectrotonus. Reflex waves can be interpolated between the tetanic ones when the frequency of the last ones is not too high. The discharge of centrifugal impulses, recorded in the ventral root, corresponds in time with the rising phase and summit of the spinal potential. The generalized synchronization is apparently the result of an electrical interaction of the active neurons. They may still beat in phase in two adjacent segments functionally separated by a complete transection of the cord.

Motoneuron synchronization has been described by Buchthal *et al.* (75) as a characteristic feature of the voluntary contraction in pathological conditions, especially in subacute and chronic poliomyelitis. The symptom, disclosed by polygraphic recording of the action potentials of different regions of the same muscle, is said to be so constant as to be of diagnostic value. For the theoretical aspects of neuronics synchronization the papers by Arvanitaki (47), and by Fessard (76, 77, 78) should be consulted.

Oscillographic study of the strychnine-tetanus (74) has afforded an opportunity to confirm the observation by Scheminsky (79) of a functional polarization of the spinal neurons, revealed

by the opposite effects of ascending and descending currents on their reflex and spontaneous activity. The significance of this phenomenon remains obscure.

The pupilloconstrictor neurons (cat) show a striking inherent tonus when they are completely deafferented by two transections of the brain stem, one placed above and the other below the oculomotor nucleus (80). The extreme myosis so produced lasts indefinitely, but yields to sympathetic efferent impulses and to atropine. It may be mentioned that the equally intense myosis, which results from a single transection placed immediately behind the oculomotor nucleus, can also be reduced by the central inhibitory effect of corticofugal and diencephalofugal impulses (after the bilateral section of the cervical sympathetic). The central inhibitory mydriases so produced (81) takes a long time to disappear. It is never as complete as the mydriasis resulting from sympathetic efferent impulses (cf. 82).

The study by Bronk & Brink (83) of the chemical activation of the sympathetic ganglion has revealed the existence of a stage of subliminal (local) and graduated oscillations of potential, of a striking regularity of frequency and sinusoidal shape, on which propagated spikes are superimposed when a critical voltage is attained. This important observation, which recalls similar ones by Adrian & Gelfan (84) on muscle fibers and by Arvanitaki (85, 86) on invertebrate nerve fibers [see also (87, 88, 89)], may be of interest for the interpretation of the sinusoidal waves of varied frequency of the human EEG. Perhaps the reluctance of physiologists to admit the description of biological phenomena by sinus functions will prove to have been unjustified.

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LABORATOIRE DE PATHOLOGIE GÉNÉRALE  
UNIVERSITÉ DE BRUXELLES  
BRUSSELS, BELGIUM

## BIOELECTRIC POTENTIALS IN THE NERVOUS SYSTEM AND IN MUSCLE<sup>1</sup>

BY HARRY GRUNDFEST

*Department of Neurology, College of Physicians and  
Surgeons, Columbia University,  
New York, New York*

The productivity of American and British electrophysiological laboratories had not yet returned to the prewar level during the past year, despite the recent development in America of a number of new laboratories. This relative decrease of output has been compensated by the activity, during the past few years, of several new centers in other countries, particularly of Granit at Stockholm, and of von Muralt at Berne; by the work of Tasaki and his colleagues in Japan; by the steady wartime productivity of Eccles, Katz, and Kuffler from the Antipodes; and of investigators such as Arvanitaki, Bremer, Fessard, and others from the European countries occupied by the Germans.

The return of free scientific intercourse among physiologists has been greatly advanced by the renewal of the annual meetings of the American Physiological Society, and by the first, quasi-international conferences on the physiology of nerve and muscle, which were held this year by the New York Academy of Sciences.<sup>2</sup> Information on the work of Soviet physiologists is still unsatisfactory. At the beginning of this decade, the Russian literature evidenced the development of intense interest and activity in the current fields of electrophysiology. Lack of modern equipment forced the Russian group of workers to draw upon ingenious, but indirect, methods for the study of phenomena more directly observed with the sophisticated methods of other investigators. The latest publications from the Beritoff laboratory (11, 12, 14, 15) indicate that modern equipment is becoming available in the Soviet

<sup>1</sup> Work published in journals available on October 1, 1946 was, as far as possible, included in this review. Because of the large list of references, it was decided to limit the number of references to older literature in giving the background for the current work. The sources in which lists of references are to be found are therefore noted in the text.

<sup>2</sup> I wish to thank the New York Academy of Sciences for making available to me advance copies of the various papers presented at the Conference on the physicochemical mechanisms of nerve activity.

Union. As the war's ravages are repaired, and if closer relations are established between Russian and non-Russian scientists, we may look forward to a school of modern electrophysiology from the country which gave us investigators of the rank of Sechenov, Wedensky, Pavlov, Samoilov, and others of earlier times.

The past year has seen a good deal of stock taking in the form of reviews. Most of the current work in general neurophysiology has been reviewed briefly by Gerard & Libet (57). Bishop (21) has summarized the present knowledge on *Neural Mechanism of Cutaneous Pain*. Prosser (117) has reviewed the field of invertebrate neurophysiology. Welsh & Schallek (149) have done the same for arthropod nervous systems. In a review on the veratrum alkaloids (82), the effects of this group of drugs on the electrical manifestations of nerve and muscle have been critically analyzed. The role of acetylcholine and of epinephrine have been presented from diverse points of view in nine reviews (36, 50, 56, 57, 94, 108 to 114). The electrical and physiological properties of the electric organ have also been reviewed (42, 51). The monograph by von Muralt (106) is sure to stimulate considerable discussion. The new edition of the classical "Howell's Textbook of Physiology" (53) contains an extensive section by Lloyd that incorporates much of the latest electrophysiological work. In addition, the appearance of a new, American edition of Höber's famous book (69) on the physical chemistry of the cell should be noted.

#### NATURE AND SITE OF THE BIOELECTRIC POTENTIAL

Recent evidence by Hodgkin & Huxley (73, 74) and by Curtis & Cole (44) as well as earlier work particularly of Cole and his collaborators has presented physiologists with data which do not fit concepts of a simple static membrane as the source of the bioelectric potential. The earlier experiments had brought in factors of membrane rectification and of inductance (39) which could be coped with, at least in a formal manner, on the basis of classical views. The problem posed by the latest work, on the other hand, requires reexamination of the entire concept wherein the membrane becomes either completely, or largely depolarized during activity, and whereby the spike represents the abolition of the resting potential in the completely depolarized membrane. Hodgkin & Huxley and Curtis & Cole have now shown, with but little likelihood of error, that the membrane of the single giant fiber during activity

becomes polarized in the opposite sense rather than merely depolarized. The evidence is based on measurements of the potential of the resting and the active nerve with one electrode inside the fiber and the other outside. Except for errors introduced by junctional potentials, these measurements represent the potential differences across the nerve membrane during rest and activity. The resting potentials thus measured averaged 41 mv. (74) and 51 mv. (44). The spike recorded from the same electrodes on stimulation of the nerve was as high as 95 mv. (74) and 168 mv. (44) in the opposite sense. Tasaki (144) has independently postulated values of 80 to 100 mv. for the spike of single medullated vertebrate nerve fibers. Hodgkin & Huxley believe that the higher values of Curtis & Cole "may have approached more closely to normal." The measurements with electrodes across the membrane of the squid giant fiber have been paralleled by similar results in single muscle fibers of the frog by Graham & Gerard (60), although the absolute potential changes during activity could not be directly determined. On the smaller single fibers of lobster and crab nerves Hodgkin & Huxley have obtained similar results with less direct measurements. The data all clearly indicate that during activity the potential developed across the membrane is not merely the abolition of the resting potential due to a transient breakdown of the polarized cell membrane, but that actually during activity the membrane becomes polarized in the reverse sense.

Hodgkin & Huxley discuss possible explanations in terms of the classical concepts of the depolarized active membrane. They conclude that: (a) it is unlikely that junctional potentials of the required magnitude (ca 50 mv.) exist to reduce the true resting potential to the observed value; (b) even if the required magnitudes of junctional potentials exist, they should be symmetrical about the depolarized membrane and should therefore cancel and contribute nothing to the spike; and (c) additional hypotheses, such as the assumption that the membrane changes from being selectively potassium permeable at rest to one which during activity is permeable to potassium and sodium but not to chloride, can be approximately checked from known data. While this assumption yields a small reversal of emf during activity, it cannot account for the many times larger changes actually observed. They therefore decide "that the difference between action and resting potentials indicates a real reversal of potential at the surface membrane."



Four types of explanation are advanced to account for the reversal of the membrane potential during activity. In the words of the authors, these are:

- (a) The active membrane becomes selectively permeable to anions which are present in the axoplasm, but are in low or zero concentration in sea water.
- (b) Activity involves a change in the orientation of dipoles in the surface membrane.
- (c) Explanation in terms of apparent membrane inductance.
- (d) Series capacity hypothesis.

The first hypothesis assumes that during activity the membrane either becomes permeable to existing anions, or the formation of a new species of anion, with high mobility through the membrane. This is rejected as unlikely, "since it is hard to imagine that the concentration or mobility of lactate or any other organic ion would be sufficient to swamp the contributions of  $K^+$  and  $Cl^-$  to the membrane potential." The different view of Höber (70) will be presented below.

The second hypothesis conceives of two sources for the bioelectric potential, one which gives rise to the resting potential, and another, produced only during activity, delivered in parallel, but opposed to the first source. The possibility of a bimolecular layer of dipoles (either lipid or protein) is specifically considered. Normally, during rest, the layer might be oriented so that the molecules lie back to back, with the nonpolar elements together, and with the negative polar groups presented equally toward the outer and inner surfaces of the membrane. In this condition, both recording electrodes would be equally affected. If, during activity, the inner layer became "deorientated in some way" or were removed, a transient wave of negativity would result. The duration of the wave would depend on the rate at which the membrane capacity was discharged by ions, and the time constant of this process should be the same as the electrical time constant of the whole membrane. Calculations indicate that "the rate of change of molecular orientation would be equivalent to that produced by a dipole layer with a p.d. of 420 mv. collapsing during a period of 0.1 msec." This is not an impossible assumption "since the p.d. arising from a fatty acid such as butyric acid has a maximum value of about 350 mv." while values in excess of 600 mv. have been recorded with other compounds.

Fessard (51) has pointed out that, for the case of single units of the electric organ, the simple polarized membrane is likewise un-

satisfactory. Like Hodgkin & Huxley, he has suggested the possibility of a polarized membrane, composed of a double layer leaflet of polar, lipid, and protein molecules. The arrangement he postulates is reversed from the one described above, the positive charges being presented to the outside and inside faces of the membrane.

The third type of explanation, already put forward by Curtis & Cole (44) but somewhat further developed by Hodgkin & Huxley, rests upon the presence of the inductive component which Cole & Baker (40) had found in the membrane of the squid giant fiber. Hodgkin & Huxley investigated the behavior of an equivalent circuit containing the membrane capacity (1.0 mF), shunted by a series network composed of the source of potential, the resting resistance of the membrane (500 ohms), and the membrane inductance (0.2 H). It was assumed that during excitation the potential source and the resting resistance are shunted by the resistance during activity (25 ohms, based on the experimental data of Cole and his co-workers), as would happen if the membrane became depolarized. The resulting surge reproduces satisfactorily the observed potentials. Hodgkin & Huxley say however, "we are reluctant to accept the idea of a genuine inductance in the membrane, as it is difficult to attach any physical significance to such a concept."

The fourth explanation offered, assumes that the emf of the resting membrane is "in series" with the membrane capacity instead of being in parallel, as classically supposed. An active region near the site of the recording electrodes would, prior to exciting the region under the electrodes, reduce the p.d. across the membrane and charge the membrane capacity to a reversed potential. During excitation of the recording region, the local potential source having collapsed in the breakdown of the membrane, the capacity would discharge to give rise to the reverse action potential, and in turn, "should be capable of reducing adjacent parts of the membrane to zero p.d. as was assumed initially" thus maintaining propagation of the impulse.

Of these explanations, the first and second introduce new dynamic concepts into the processes of the classical membrane theory while the last two modify the structural concepts. The present reviewer confesses to a reluctance like that of Hodgkin & Huxley "to accept the idea of a genuine inductance in the membrane," though without questioning the basic information on which the formal

identification of an inductive circuit has been established. Such formally inductive circuits might perhaps be interpreted by physicochemical systems in a dynamically conceived, metabolically maintained membrane, and they find parallels in electrochemical systems (20).

The reviewer wonders if the series capacity hypothesis may not find another, less drastic form. It is known that the myelin sheath, which is present in all nerves to greater or lesser degree, is a polarizable structure (146). We would thus appear to have a capacity in series with the recording electrodes, and with the standard network of the nerve equivalent circuit.

Höber (70) in a beautifully concise paper has discussed the first hypothesis of Hodgkin & Huxley. With reference first to the injury potential, Höber points out that, while changes in the concentration of inorganic cations or anions produce relatively small reductions in the injury potential, organic anions, particularly those with polar structure, like the higher fatty acids, can easily reverse the injury potential and produce strong effects upon the elements of the colloidal structure of the membrane. For the same reasons as do Hodgkin & Huxley, Höber also rejects the possibility that lactate ions released during activity play a role in the production of the spike, but he calls attention to the "organic nonpolar-polar, hydrophobic-hydrophylic anions" which might "be liberated as the excitation wave travels along the fiber." Over and above acetylcholine which will be discussed later, there are numerous possibilities for the liberation of these active organic anions. Höber suggests that they might be liberated through a number of ways, for example by the action of potassium ions, or enzymatically. If and when released, the organic anion (s) might act directly through intrinsic electrical forces (Hodgkin & Huxley, explanation *a*) or, if systems such as the oriented dipole layers (of explanation *b*) be present, might cause further changes in the configuration of the membrane structure and thus add to the magnitude of the reversal of the potential.

The reviewer is tempted, though with diffidence, to add some observations on this problem. It must be remembered that the excitable membranes of nervous tissue and of muscle function in the presence of very high potential fields. Assuming a p.d. of 1 mv. drop for each  $A^\circ$  of the membrane, the total field is equivalent to  $10^5$  volts per cm. To the reviewer's knowledge, no data are availa-

ble regarding the behavior of even simple barrier systems under electrical stresses of such magnitude. Furthermore, it is unlikely that during passage of activity along an excitable tissue such as nerve or muscle, the system will have reached anything like equilibrium. The theory of membrane potentials has recently been extended by Teorell and Meyer & Sievers (see the discussion and bibliography in Höber *et al.* [(69), pp. 71-72] to apply to some of these nonequilibrium systems. According to the Teorell-Meyer & Sievers theory, the membrane potential is a complex derived from mobile and immobile ions within the membrane. Opposed and unequal Donnan potentials exist at the two surfaces of the membrane. Further, the interior of the membrane is the seat of a diffusion potential produced by the concentration gradients of the mobile ions. The whole membrane potential is the sum of the diffusion potential and of the two, oppositely directed Donnan potentials. Meyer has suggested that bioelectric phenomena may be the resultant of the existence and morphological arrangement of both cation and anion selective membranes, as well as of chemical reactions which may liberate or capture dissolved ions. He has recently (102) pursued this idea by very ingenious methods toward analyzing the composition and activities of the various layers of the frog's skin. Other investigators are also currently attempting to analyze the electrical properties and their functional correlations in these organ tissues (118, 119, 141, 148). While the functional complexity of the tissue membrane probably does not exist in the excitable membranes of nerve and muscle, there is no longer sufficient reason to assume simplicity of structure, now that classical equivalent circuits have to be considerably modified because of the new evidence of Cole, Hodgkin, and their co-workers. The dynamical concepts of bioelectric potentials developed by Hodgkin & Huxley, by Höber, and by the Teorell-Meyer & Sievers theory will no doubt stimulate better understanding of the membranes of excitable tissues.

The consequences of such dynamic formulations require some further observations. As factors in the bioelectric potential, theories involving chemical changes will have to conform to relatively invariant, and quite brief time limits which are well known or easily obtained for nerve and muscle through physical measurements. Thus far, only Nachmansohn (see below) in his studies on the acetylcholine-cholinesterase system, has paid particular attention

to this feature. Furthermore, the study of chemical processes which may enter into the development of the bioelectric potential is at present hampered by the absence of rapid methods commensurate with the speed of the development and subsidence of the potential. Pioneer work is being done by von Muralt in attempting to develop such methods. These are described in his monograph (106) together with some of the early results.

Shanes (132 to 134)<sup>3</sup> has applied some interesting methods to the study of the linkage between metabolic processes and the resting potential, a work which is the outgrowth of earlier experiments by Shanes & Brown (131). The new experiments examine principally the effects of sulfanilamide and other inhibitors of carbonic anhydrase upon the course of the resting potentials of frog (132, 133) and crab (134) nerve when these potentials are modified by high external carbon dioxide or anoxia. The results appear consistent with the hypothesis that about 50 per cent of the resting potential is contributed through the exchange of hydrogen ions, formed as a result of the metabolic production of carbon dioxide, for external potassium ions. The other portion of the resting potential is presumably contributed by a Donnan potential. The exchange of internal  $H^+$  for external  $K^+$  (a possible basis for the "potassium pump") is affected not only by internal factors such as the interference with enzymatic steps in the metabolism, but by external factors such as the ionic environment. The rather different relations for the resting potentials of frog and crab nerves are ascribed to different permeabilities of their membranes and to differences in metabolism, crab nerve having an extremely high glycogen content and consumption.

That the bioelectric potential is more complex than can be accounted for by a simple boundary membrane is further indicated in the recently available work of Arvanitaki [(6), which also gives a complete bibliography of her past work]. At the anode of a stimulus applied to the giant fiber, there appears a positive prepotential. It can be made oscillatory by calcium deficiency, and the resulting potential sequence and its properties can be homologized with that part of the oscillatory response at the cathode which is subsequent to the first negativity. The excitability at the anode follows the potential cycle, being lowered during development of the positive,

<sup>3</sup> Manuscripts of unpublished work have been kindly made available to me by Dr. Shanes.

and heightened during the swing to the negative prepotentials. Propagated impulses may arise at the anode if the local oscillations build up to a sufficiently high negative level.

Another important feature is the fact that the temperature coefficients of the initial anodal positivity and of the subsequent negative phase are different. The critical thermal increments are 12,000 and 8,000 cal. per mol. respectively. These correspond, in reverse sense, to the values for the first two components of the oscillatory potential which develops at the cathode (38), and this correspondence indicates that the positive and negative prepotentials arise from the involvement of different, but linked reaction chains, one of which is first set into motion by cathodal, and the other by anodal currents.

The existence of similar oscillatory phenomena in vertebrate nerves has been clearly indicated in the prewar experiments of Brink & Bronk (28) who have given us a summary of that work as well as an analysis of chemical excitation of nerve. In this connection it must be recalled that Taski (145) has reported failure to find evidence for a local response in single nerve fibers of the toad.

Von Muralt, on the basis of his own optical investigations of the structure of frog single nerve fibers, and on the work of Tasaki & Takeuchi (143, 147), has presented (106) a revolutionary picture of the nerve membrane. According to this view, the excitable membrane is transverse to the fiber, is located at the node of Ranvier, and closes off each internodal segment from its neighbors. Current flow through this membrane excites the entire segment and propagation takes place in a "saltatory" fashion as postulated earlier by Erlanger & Blair (49).

The validity or the productiveness of this hypothesis is still to be tested. The ultraviolet and polarization microscope pictures given in von Muralt's book show what appears to be a membrane but earlier histological reports stated that neurofibrils penetrate the membrane. The validity of the hypothesis will therefore depend on demonstrating that, if the membrane does exist, it forms an ion selective and polarizable structure. The septal membranes of invertebrate giant fibers do not appear to form such polarizable barriers (30, 126, 127).

#### EXCITATION, PROPAGATION, AND TRANSMISSION

*Excitation by electrical stimuli.*—With the exception of the ac-

tive phenomenon at the anode described by Arvanitaki (6), there have been no new concepts introduced into this problem. The use of electrical stimuli of different forms in attempts to check experimentally the various theories of the excitatory process discussed in the older monographs of Katz (78) and Schaefer (128) have not proved conspicuously successful. With linearly rising currents sensory nerves accommodate less than do motor nerves in animals (138) and in the human subject (87). Ischemia increases accommodation in human nerves, but for a considerable period after release of the ischemic block, there is virtual abolition of accommodation. Linearly rising currents have been used successfully to stimulate selectively motor units in man (88, 89). Comparison of electrical activity evoked by direct stimulation of the nerve and by voluntary movements discloses some interesting results. The order of activation in both cases is the same. The first to respond are units giving small potentials. The frequencies of repetitive activity are also about the same.

Renewed attempts to find an excitability curve specific for the muscle end plate (84) have failed to disclose one in the single nerve muscle fiber preparation. In the course of this work, however, Kuffler has confirmed the older finding (64) that curarine, in paralytic and larger doses, does not affect the excitability curve of the muscle fiber.

The choice of electrical stimuli for general use (particularly for electroshock therapy) is discussed from a theoretical view by Ofner (116). By employing the simplest of the excitation equations he calculates that the most efficient stimulus (in terms of least power which would be consumed) should have an exponential rise. The next most effective should be a square pulse, which would require about 22 per cent more power. Exponentially falling stimuli should require about 85 per cent more power, and sinusoidal stimuli several times more. Since square waves are most easily generated of the more efficient stimuli, these are recommended in preference to a.c. for clinical use. It should be pointed out that the excitation equation employed is valid only for very brief stimuli (less than 1.0 msec.) and the calculation may or may not be valid for 60 cycle stimuli.

*The local excitatory process.*—Arvanitaki, in the paper discussed above (6), shows that a stimulus applied to a nerve which is already in a state of local oscillations will vary in effectiveness, de-



pending on whether the stimulus (whatever its sign) tends to develop a new local potential in the presence of a similarly or oppositely signed phase of the oscillations. In-phase potentials will augment while out of phase potentials will depress the effect of the stimulus. This finding has particular bearing on the "optimum frequency" of stimulation with a.c. This frequency should be such that its half period corresponds with the period of the local potentials.

Two papers by Tasaki (144, 145) deal with the local excitatory process. Some of this work is a confirmation, with new and precise methods, of older data. Subthreshold stimuli of increasing strength, up to 60 per cent of threshold, give rise to a proportionally increasing local excitatory process. Failure of proportionality with higher intensities of stimulus is not explained, but one wonders if it may not be an indication of a local response, as observed by Katz (77) and Hodgkin (72).

Tasaki could find no post cathodal depression under the conditions of his experiments. He ascribes depression to the presence of the sheath. It will be recalled that no depression has been found in muscle fibers which are bathed in fluid (65). The local excitatory process decays to zero in approximately 0.4 msec. The excitatory effects produced by two weak stimuli of either sign sum algebraically. The effects of two stimuli applied through different electrodes also sum, but their magnitudes are reduced and their time course is distorted because of their conduction electrotonically.

With his method of nodal blocking Tasaki finds, as did Hodgkin (71), that the spike which is propagated to the block sets up there a local excitatory process. The effect of the blocked potential is conducted electrotonically to successive nodes beyond the block, decaying exponentially, with a loss of excitatory effectiveness of one half for each segment.

*The initiation of the action and its propagation.*—Earlier reviews in this series have signalized the basic advances which have been made in these and related problems. During the flow of a current which initiates the action, the resistance of the membrane falls very greatly, and a local circuit is established with current flowing out of neighboring regions of the membrane into the depolarized, or reversely polarized, excited part of the membrane. This outward flow of current sets up excitatory changes, which may be accompanied, at least in invertebrate nerves, by a local response.

Some data on the constants of the local circuit have been given by Tasaki & Takeuchi (146) for their single fiber preparation and reviewed by Renshaw (121). The spike recorded from these single fibers is characterized by a very steep rise. The time to the crest is about one-tenth of the total duration, whereas the value is about one-third for mammalian fibers (54). The latter measurements were, however, carried out in multifiber preparations, and may have been distorted by the presence of polarizable tissues. The spike duration (1.5 to 2.0 msec.) found by Tasaki & Takeuchi is within the standard range for cold blooded vertebrates. The "safety factor" of the spike is about five. The greater vulnerability of the node, as compared with the internodal stretch, to narcosis and to injury, reported earlier by the Japanese workers, is paralleled by the greater susceptibility of the node to damage by ultraviolet radiation (75).

By combining narcosis and polarization of nodes, Tasaki & Takeuchi (143, 147) observe, as did Erlanger & Blair (49), varieties of compounded spikes which they too interpret as indicating that each internodal segment responds as a unit, with each subsequent activation arising at the next following node after a slight delay, normally about 0.1 msec. Until more physiologists have become acquainted with this type of preparation and its properties, this reviewer deems caution the better course in interpreting the published records, particularly since some of the circuits used are complex and might lead to a variety of artefacts (125).

One doubting comment is, of course, obvious. Fibers with nodes are not the only ones known. While the invertebrate nerve fibers and the unmyelinated ones of vertebrates, none of which contain nodes, might be placed in a special category because of their slow conduction velocities, the tracts of the spinal cord also consist of myelinated fibers which do not have nodes, and which nevertheless are rapidly conducting. Thus, the dorsal spinocerebellar fibers conduct with velocities of 140 mps. or more (68). Their high velocities can be accounted for entirely on the basis of large fiber diameters. On the basis of experimental evidence, therefore, there does not appear to be any difference in the conductive ability of fibers with, and without nodes.

In the selachians, the cells of the dorsal root ganglia are bipolar. Impulses arising at either part of the axon go through the cell without complications (37).

*Chemical intermediates in nerve action.*—Several hypotheses have been put forward regarding the participation of specific chemical substances in the action potential. Von Muralt (106) has brought together the evidence for at least three such "action substances," acetylcholine, thiamin ("aneurin"), and potassium. The data thus far reported will need considerable amplification before their significance can be properly judged. A very small quantity of acetylcholine appears to be liberated in nerve during the passage of the impulse (104). About ten times as much thiamin (vitamin B<sub>1</sub>) which is located in the sheath is likewise formed (107). Von Muralt believes (105) "that acetylcholine formation is essential for the excitation or recovery process and that aneurin is a reservoir substance closely connected with the formation and disappearance of acetylcholine."

Gesell (see earlier reviews) has proposed what he calls the "electrotonic theory" (58) which, in the special case, at least, of synaptic transmission links hydrogen ions, acetylcholine, and depolarization phenomena. Additional preliminary papers elaborating these views have appeared (26, 52, 59, 137).

Other brief publications have appeared describing experiments on models in support of the view that the action potential of nerve is a manifestation of the phase boundary potential type, and that it is produced by the generation of acetylcholine on one side of a membrane and subsides when the substance has migrated so that both sides of the membrane are equally affected (8, 9, 19).

During the past few years, Nachmansohn has reported a variety of experiments which appear to establish a linkage between the acetylcholine system and electrical activity in nerve, muscle, and electric organ. He has also proposed a view which is essentially a chemical modification of the electrical theory (reviewed in 108 to 114). It should be pointed out that this hypothesis denies that transmission of the impulse is accomplished by a chemical mediator which is released at the synapse. Von Muralt (106) apparently still believes that acetylcholine is a mediator in the above sense.

According to Nachmansohn's hypothesis, the initiating and propagating agent for the impulse is, as in classical theory, the flow of current through the membrane. However, it is suggested that the flow of current out of an inactive region of the membrane may act upon an unknown precursor, to liberate acetylcholine in that region, which, then depolarizes the membrane. A new local circuit

is now established and causes propagation of the impulse. Nachmansohn has taken into consideration the requirement of the time relations of the action. Since the entire cycle of the spike may last as little as 0.4 msec. and may be repeated at frequencies higher than 1,000 per sec., the rapid liberation and rapid destruction of the acetylcholine must be demonstrated. At least the second condition appears to be satisfied, because of the presence in all types of nerves (34) and in muscle of a rapidly acting enzyme, cholinesterase, which is relatively specific in hydrolyzing acetylcholine. In the giant fiber of the squid, the enzyme is concentrated in the surface of the fiber, presumably at or near the excitable membrane. Another enzyme is also described which synthesizes acetylcholine. Nachmansohn has developed a schema, which elaborates the place of the ester in the metabolic cycle.

By making assumptions concerning the relation between the presence and activity of the hydrolyzing enzyme and those of the ester, Nachmansohn has gathered an impressive body of indirect evidence for his hypothesis. Only the recent papers dealing with this will be discussed.

Physostigmine and strychnine, both of which inhibit cholinesterase reversibly, have been shown (35) to abolish the action potential of squid nerve fibers reversibly. Physostigmine acts likewise on the sciatic nerve of the bullfrog, (unpublished) reports to the contrary (98) notwithstanding. The failure of prostigmine, which *in vitro* is also a strong anticholinesterase, to act in the same way has been explained as due to its quaternary ammonium structure containing three methyl radicals and its consequent failure to enter the nerve or muscle (34). The structural similarity of prostigmine and acetylcholine itself have in turn been used to explain the failure of high concentration of acetylcholine in the bathing medium (98) to affect the nerve.

The recent availability of another powerful inhibitor of cholinesterase, di-isopropyl fluorophosphate (DFP), appeared to offer a direct, crucial test of Nachmansohn's hypothesis, because DFP can inactivate cholinesterase irreversibly. In experiments with the sciatic nerve of the bullfrog (43), it was found that the action potential was abolished by applying DFP, but reappeared on removal of the substance. Measurements of the cholinesterase activity of nerves taken after some time from animals injected with

massive doses of DFP indicated only traces of cholinesterase.

These results and their implications have been rejected (32, 33) on the basis of an extensive series of experiments, especially on the giant and other nerves of the squid, and the giant fibers of the abdominal cord of the lobster. It was found that the complete return of the action potentials in these nerves is always accompanied by the presence of at least 20 per cent of the initial cholinesterase (or, what might be called a chemical safety factor of five). The return of this amount of cholinesterase was explained by the demonstration that the action of DFP is not immediately an irreversible one, and that the degree of irreversibility depends on time and temperature. The time dependence of the inactivation process was used to demonstrate that progressive irreversibility of the action potential was paralleled by a progressive decrease in the available cholinesterase (32). Other experiments use the temperature dependence to show that at low temperatures, the amount of cholinesterase which remains under otherwise similar conditions is increased from 20 per cent at 20 to 25°C to approximately 50 per cent at 5 to 7°C. (33).

A number of additional factors, such as the small initial concentration of cholinesterase in the sciatic nerve of the bullfrog, the demonstration that appreciable inhibition of cholinesterase may occur during manometric measurements because of small quantities of DFP retained in the nerve, and technical factors peculiar to the manometric method (carbon dioxide retention by protein, and absorption by liberated base) would, it is pointed out (33), bring the determination of cholinesterase in the nerves of DFP poisoned bullfrogs below the limit of the manometric method. The presence of cholinesterase in such nerves is shown, however, with the frog rectus abdominis bioassay, though quantitative comparison as to the concentration required for function with the data obtained on more favorable preparations is precluded.

Because the inactivation of cholinesterase by DFP is not immediately irreversible, this crucial experiment against the theory has not materialized. The experiments with anticholinesterases do, however, strongly suggest that the acetylcholine-cholinesterase system enters quite intimately into the elicitation of the propagated action potential, not only of nerve but also of muscle (41, 34). Of special interest is the demonstration that sensory and adre-

nergic nerves, like motor nerves and muscle, are similarly affected by the anticholinesterases and that they contain specific cholinesterase (34).

The rapidity of the action of substances which are relatively specific inhibitors of cholinesterase on the potentials of different kinds of nerves and of muscle (41, 34) and the correlation of the degree of the reversibility of the effect with the degree of reversibility of the inhibition of cholinesterase, therefore make likely the conclusion (34) that, whatever its specific role may be, the acetylcholine system enters into the activity of excitable conductile tissues at a more crucial stage than do, for example, oxygen and other metabolic systems.

The reviewer wishes to stress, however, that the experiments just discussed do no more than this. They neither give us information as to the precise point at which acetylcholine enters the cycle of activity nor what it does. The hypothesis that acetylcholine enters specifically into the earliest steps of the cycle, to depolarize the membrane, thus far has received no direct test. The data available at present, tell nothing about the internal action of acetylcholine. For example, unless subsidiary hypotheses are made, the linear relation which has been found (111) between the relative concentration of cholinesterase and the voltage developed in the unitary elements of the electric organ relates the electric effect not with the presumed electrogenic agent but with its inactivator. It would be desirable to subject the hypothesis to several or all of the following experimental tests which would show whether:

(a) The postulated formation of acetylcholine from its precursor takes place at the required time, (b) the acetylcholine presumed to be liberated at the axonal membrane exerts the effects that are postulated, and (c) the time relations of the electrical activity can be modified predictably by experimental changes in the acetylcholine system.

This last type of test is of particular importance for the electrophysiological aspects of the theory. Thus far, the theory has inserted an extra step into the generally conceived mechanism of excitation and conduction, but has not contributed to an explanation of the mechanism. Indeed, as at present stated, it does not even deal with the more subtle phenomena which have come to light in recent years and some of which are discussed in this review. While, therefore, it has helped to throw light on some aspects of the chemistry of nerve activity and while it offers a possible inte-

gration of the chemical and electrical aspects of electrophysiology, the theory that acetylcholine depolarizes the membrane must still meet the test of necessity. The reader is reminded, however, that currently even the more purely electrical approach is considering the possible role of organic anions.

A vigorous attack upon all phases of the hypothesis and an equally vigorous reply will be found in the discussions by Gerard and Nachmansohn before the New York Academy of Sciences (56, 110).

*Transmission of the impulse across a junction.*—The background of the different views on this problem will be found in the earlier of this series of reviews on this subject and will be omitted here. In the first review of this series [(29), p. 398] it was possible to say that "the most generally accepted explanation of synaptic transmission assumes that the presynaptic impulse liberates a small amount of acetylcholine, and this in turn sets up the excitatory process in the cell body." The shift of opinion among physiologists was signalized in the second (66) and subsequent reviews.

The reported and disputed liberation of acetylcholine during synaptic transmission can be explained on the basis of the view that the substance enters intimately into the development of the electrical activity. If acetylcholine were liberated during the activity of nerve, an assumption which is common to a number of the views we have discussed earlier, minute quantities such as have been reported would inevitably get through the barrier formed by the membrane and the cholinesterase (109). Although the specific excitatory effects of acetylcholine, and, as Loewi (94) has pointed out, the inhibitory effects on some tissues produced by stimulation of nerves have not yet been satisfactorily explained, Eccles, who has made a number of attempts to bring the neurohumoral theory into agreement with the electrical theory has stated (47) that "it is unsatisfactory that the acetylcholine hypothesis has had to be reconciled with new experimental evidence, by . . . making subsidiary *ad hoc* hypotheses, which had not been independently testable."

Two recent papers which compare the excitatory effects produced on muscle by stimulation of their nerves and by acetylcholine deny that the mechanism of neuromuscular transmission is identical with the drug effect, and therefore oppose the neurohumoral theory. Under various conditions, muscles which are



easily stimulated through their nerves give little, or no mechanical response to acetylcholine (129, 138).

The more recent attempts to elaborate an electrical theory of synaptic transmission have been noted in detail in earlier reviews of this series [see particularly that by Bishop, (20)].

(a) Hodgkin (71) produced a functional discontinuity in nerve (by cold) and studied the excitatory effect of the action potential conducted to the block, on the region beyond. By a modification of the method, using cocaine to produce the block, Lorente de N6 (97) extended this study.

(b) The old observation of Hering that at the cut end of a nerve impulses arriving in some fibers can set up activity in other fibers was later used by Jasper & Monnier (76), and by Renshaw & Thermann (124), and recently elaborated by Granit and his co-workers (61), who have used it as a model of the synapse and of the sensory receptor (17). Two recent papers by Skoglund compare the excitatory effects produced in post-ephaptic<sup>4</sup> fibers by pre-ephaptic activity and by electrical stimulation (139) and study the effects of polarization on the ephapse (140).

(c) A different approach arose from the work of Katz & Schmitt (79, 80) who described quantitatively the excitatory effects produced in one fiber by the action currents flowing out of an adjacent active fiber. These results were elaborated by Blair & Erlanger (22) and by Marazzi & Lorente de N6 (100).

(d) A more generalized method was developed by Arvanitaki (5) who studied the effects of activity in one giant fiber on another, unstimulated nerve, when the two were oriented in different ways relative to one another.

These methods of creating and studying the ephapse have yielded a qualitatively but not quantitatively satisfactory picture. The current of action of one fiber, the pre-element, can produce either anodal or cathodal polarization in the other, the postfiber, depending on the time and geometrical relations. In the simplest case, that of two parallel and contiguous fibers, the first effect is that produced by an anodal current and is depressive during the period when the active region of the pre-fiber is approaching the test region of the inactive post-element. When the activity reaches the test region, the flow of current is reversed and increased excitability is observed. This is again followed by an anodal, depressed stage when the activity is traveling away. The time relations are given accurately in this case. Different geometric relations can give other effects predictable in direction, and derivable from the simple case, but they can be complicated in various ways.

<sup>4</sup> The reviewer prefers the use of this term, coined by Arvanitaki (5) to the use of "transsynaptic."

The models do not, however, give (a) A precise explanation of synaptic delay, although Arvanitaki (6) and Schoepfle (130) have pointed out that the first, anodal polarization may cause the delay, perhaps by depressing excitability for a period, or (b) Only in a few situations is it possible for the pre-element to stimulate the post-element. The excitability effects developed in the latter generally are only about one-fifth or one-tenth of threshold.

Eccles (45, 47) has now developed a general hypothesis of synaptic and neuromuscular transmission which approaches the goal of yielding quantitative answers. In this hypothesis, he has assumed the following conditions.

(a) The geometry of the synapse may be considered to consist of a presynaptic fiber ending represented as a cylindrical membrane, its closed end in apposition to a large plane surface membrane which represents the postsynaptic cell. The conditions of the flow of current are those which are known for the ephapse. At first there is anodal and then cathodal current flow into the postsynaptic membrane.

(b) In general, the surface membranes have the properties that are found in peripheral nerves, and can be represented by the same circuit elements: resistance, capacity, rectifier action, and a source of emf. The same dynamic modifications of the potentials will occur as in nerve.

(c) The synaptic region of the postsynaptic cell has a unique electrical and physiological property in that cathodal polarization sets up a graduated, brief, local response that is accompanied by a temporarily irreversible, large diminution of the emf, but which is not the all-or-nothing "breakdown" characteristic of the propagated response.

The first two assumptions are essentially those of all electrical theories, and need not be discussed here, although Eccles' skillful use of dynamic concepts should be pointed out. The third assumption, that of a synaptic local response, such as reported in the end plate of muscle (83), solves one difficulty of the various types of ephaptic models described earlier, namely that ephaptic excitation of the postelement is relatively weak. It provides a means to amplify the stimulus received from the presynaptic potential into an effective stimulus for postsynaptic propagation. The absence of "all-or-nothing" behavior can be used to explain properties of iterative facilitation as well as inhibition. Arvanitaki (6) has likewise suggested that a local response may play an active role in synaptic transmission, with particular reference to phenomena of facilitation and inhibition.

Once the local response is produced, a catelectrotonic potential

is supposed to spread over the cell, giving the electrical responses recorded from end plates, ganglia, and cells of the central nervous system. The bibliography is too large to enumerate here. It can be found in earlier reviews and in the paper by Eccles which is under discussion (47) and in two new publications, one of which deals with the synaptic potentials of motoneurons (46), and the other with electrotonic dorsal root potentials (48). The latter applies the new hypothesis in specific situations.

Eccles envisages the sequence of events in synaptic transmission as follows:

(a) The impulse arriving in the presynaptic fiber generates a current, which gives a diphasic effect at the synaptic region of the cell. The total duration of this effect is of the order of the duration of the presynaptic spike, but somewhat prolonged by the capacities of the system. Initially, there is an anodal focus (rather large) with a cathodal surround, followed by a more localized, more intense cathodal focus, with an anodal surround. The size and intensities of the two foci are functions of the rectifier properties of the membrane.

(b) This cathodal focus sets up a brief and intense local response at the synaptic region, which has an active development and a passive decay.

(c) From this local response, a catelectrotonus spreads decrementally over the cell membrane. This is the synaptic potential.

(d) A propagated impulse is set up, if the catelectrotonus is above a critical value. If it is below that value, the catelectrotonus decays passively as the local response subsides.

Eccles has applied the hypothesis to a number of specific properties of the synapse.

(a) Irreversibility of synaptic transmission may be explained by the noncommutative sequences of anodal and cathodal effects which would be the result of asymmetric geometrical relations of the pre- and postelements. In addition, the postulate of the local response, as pointed out earlier, makes for another asymmetrical relation between the intensities of excitation produced by dromic and antidromic stimulation. Synaptic irreversibility is, however, well honored in the breach. As Eccles points out, these cases of reversibility may be explained on the basis of the hypothesis.

(b) Synaptic delay is explained as the interval from the onset of the first (anodal) changes at the membrane until the development of sufficient catelectrotonic synaptic potential to cause the initiation of the propagated impulse. In a measure, this explanation has much in common with earlier formulations. Its new aspects are the ability to give the correct order for the magnitude of the delay and to provide a simple explanation for experimentally induced shortening of the delay (95, 96).

While the hypothesis encounters certain difficulties, which Ec-

cles discusses, these are relatively minor, and arise chiefly from studies of drug effects. The testing of the theory by means of these difficulties, and by the various tests which Eccles proposes should provide stimulation for considerable new work. As this review has tried to point out, Eccles' hypothesis is essentially a synthesis of the views formulated in the past few years by many physiologists (20) and as such is built on a firm structure.

A new synaptic system, which consists of the single pre- and postsynaptic giant axons of the squid (31) will no doubt offer valuable opportunities for further tests of the hypothesis. On stimulating the preganglionic fiber and recording from the stellar ganglion, there appears after a delay, a "local response" on which is superimposed the propagated spike. Repetitive stimulation causes a decrease of this synaptic potential bringing, first, a delayed appearance of the spike, and then, its failure, so that the synaptic potential alone can be recorded. In addition to the fact that this preparation represents the activity of only two units, two other features are of value. The termination of the preganglionic fiber is of large diameter and it makes connection, not with the cell bodies of the post-fiber, but with a stretch of the fiber itself. Transmission is nevertheless in one direction only.

Of further use in studying the properties of the synapse may be the newly described giant cells of gastropods (7) which can be extruded from the ganglion in rather bizarre fashion, through the axoplasm of their fibers and which nevertheless are capable of giving potentials. The success of the work with giant axons of the squid, combined now with the new data on its synaptic system, is prompting the search for other such systems. A new giant fiber, which has a diameter of a millimeter, has been described in *Myxicola* (115).

Beritoff (12) describes interesting properties for the nervous system of the leech. Fibers can stimulate other nerve fibers, and inhibition by field currents (see a subsequent section) set up in cell masses is used to explain some of the properties of the system.

#### OTHER ASPECTS OF SYNAPTIC ACTIVITY IN THE NERVOUS SYSTEM

*The catelectrotonic potentials.*—Since the work of Barron & Matthews (10) there have been many studies of potentials recorded by using the spinal roots as "inactive" leads to derive the activities

of elements buried within the cord. In the case of electrotonic potentials recorded from the dorsal roots, two types of explanation have been offered: (a) that the potential is the index of a prolonged activity in the terminations of the roots themselves (10) and (b) that the potential represents a depolarization in the dorsal roots produced by activity in transsynaptic elements (23). Eccles has returned to this problem (48) and, in the light of his hypothesis, has given an explanation which is essentially the second, but also contains an element of the first. The synaptic response produced in the postsynaptic cell on stimulating a dorsal root, sets up a cathodal focus in the terminal membrane of the same and other dorsal root fibers. This, in turn, causes a local response in the fibers which is subsequently propagated electrotonically to be recorded at the roots. The experiments were performed on frogs, but in view of the description (46) of a synaptic potential in the motoneurons of the cat, the same explanation, with different time constants, should hold in the latter animal. The time relations are satisfactorily given by the theory only for the catelectrotonic potentials which are produced dromically, but not for those caused by antidromically induced activity of the motoneurons.

*Excitation by field currents.*—Currents flowing out of active elements are known to produce excitation in other, inactive elements (see preceding sections of this chapter). Massive excitatory effects have been reported by Libet & Gerard (90). Similar effects have been observed (27) in cats, particularly after strychninization. Stretches of the spinal cord anatomically isolated by transection will, nevertheless, show synchronization of the intense electrical activity which the drug sets up.

*Inhibition by field currents.*—In an earlier chapter of this series (66) this reviewer had suggested that the field currents set up by cell potentials (specifically, by antidromically activated motoneurons) should produce anodal polarization in some of the properly oriented presynaptic terminations, so that dromic conduction into these terminations might be blocked. Experiments were projected to test this suggestion and its consequences. They were carried out, together with a variety of others, by Renshaw (120). As was expected, it was found that antidromically induced activity in a pool of motoneurons could produce profound inhibitory, as well as facilitatory effects which could be demonstrated in other, previously inactive motoneurons. It was not known at that time

that Beritoff (13) had already demonstrated the inhibitory effects (see also 12). The experimental data thus far obtained could not distinguish whether the effect of the field current was produced on the presynaptic terminations to, or on the synaptic membranes of the tested motoneurons. Renshaw (123) has now brought forward evidence which he believes shows that impulses which arrive via one dorsal root cause, not only inhibition of the motoneuron response to a volley from another dorsal root (91), but also modify the electrical response in the fine terminations of the sensory fibers of the latter. This interpretation may have to be re-examined in the light of Eccles' hypothesis (47). The potential ascribed to the "terminal portions of the sensory fibers" falls within the synaptic delay of the motoneuron, and occurs as an elevation between that which represents the arrival of the sensory impulse at the motoneuron, and the response of the latter. Although the system is a complicated one, it may be that Renshaw has succeeded in recording in this elevation the synaptic potential which Eccles (46) finds in the motoneurons.

*Organization of activity within the spinal cord.*—In a deceptively brief paper, Lloyd (93) has analyzed the relations between the discharge zone and subliminal fringe in a pool of motoneurons, when the latter is supplied by a homogeneous presynaptic volley. As the afferent stimulus is increased (measured by the size of the dorsal root volley) the excited zone increases. But the first increase takes place in the subliminal fringe (as tested by the facilitatory effects on the response to another afferent volley). Only after the excited zone has reached a value of about 40 per cent does the discharge zone begin to grow (as measured by the response to the single, conditioning volley). The form of the relation between the maximum excited zone and the discharge zone indicates that the activation of the motoneurons proceeds partly by gradual increase of their excitability (which increases the size of the subliminal fringe) through the summation of excitatory effects produced by synaptic knobs spread over the surfaces of the cells. But this effect is not uniform for all motoneurons and, as the stimulus intensity is increased, an increasing number receive sufficient presynaptic activation to discharge them forthright.

Renshaw (122) finds that antidromic stimulation of a ventral root produces spikes in the ventral horn which persist, but in progressively decreasing numbers, for 30 to 50 msec. These appear to

be due to activity of interneurons, although these discharges do not, in turn, react upon the motoneurons. These units sometimes present a higher threshold for afferent excitation, respond to the antidromic volley with a latency of 0.7 msec., have a high rate of discharge (1,500 per sec.), and their activity may be augmented or decreased by a conditioning volley. The method of their activation is not clear. It could be either through recurrent collaterals or by means of field currents. On the basis of these results, conclusions are drawn regarding some possible properties of all interneurons, particularly on the ability to respond rapidly. Such evidence on dromically excited second order neurons, and on their conditioning has already been presented (68). Localization of certain peroneal premotor interneurons (18) which produce the later part of the spike in a motor response and data which indicate that these are activated by a complex chain (16) are reported in two papers from Granit's laboratory. The different pathways of the chain undergo cyclical changes in excitability which are analyzed.

The growing usefulness of electrophysiological methods in mapping structure and function of the central nervous system is illustrated in the results presented in a number of papers (1 to 4, 14, 15, 99, 103, 135, 142). Of special significance is the demonstration by Woolsey (150) of dual areas for the major sensory systems and of a hitherto unknown ascending tract activating the inferior olive (78).

*Electrical effects at the crustacean neuro-myal junction.*—The effects of motor and inhibitory fibers on the crustacean neuromyal junction have been reported in two new papers by Katz & Kuffler. Stimulation of the motor nerve (81) may produce a large end plate potential (e.p.p.) which leads to the onset of a propagated spike, and of a propagated muscle twitch. Nerve impulses may, however, produce a small e.p.p., which will require facilitation by repetitive stimulation to produce the propagated disturbance. The small e.p.p.'s are of themselves associated with a local contracture which is not accompanied by a spike. These contracture effects in the crustacean muscle have now been paralleled by observations on potentials associated with the contracture of frog muscle fibers (85).

Stimulation of the inhibitory nerve of crustacea (86) produces no detectable effect on the muscle fiber, but, by decreasing the e.p.p., it prevents either the propagated muscle response or the local contracture. The facilitatory process in the e.p.p. caused by



repetitive motor nerve stimulation is not affected by stimulating the inhibitory nerve. Although the e.p.p. is decreased or abolished during simultaneous stimulation of the two nerves, once the inhibitory stimulus ends, the potential rises to the height it would have had if the motor nerve only had been stimulated. This inhibitor action is said to have a drug-like action, although the drug, if such it be, is unknown. It is distinct from a second action of the inhibitory impulse, which can prevent the muscle twitch, without changing the e.p.p. (101). The latter is believed to be a direct action on the contractile process of the muscle at the junction, while the first effect is on "the junctional receptors of the muscle membrane."

*Potentials of smooth muscle.*—Bozler (24, 25) is continuing his valuable work on the potentials of the complicated electrical system which smooth muscle presents. Differential leads record a potential which has characteristics similar to those of the heart, and is identical with that of other visceral smooth muscle. The monophasic response is a long sustained negativity. Epinephrine shortens the R-T interval, but only in high concentrations does it stop activity. Premature waves can be set up soon after the T wave. The data support the view that visceral smooth muscle is a syncytial system. Spikes are seen but rarely. In the cat they appear during moderate to strong peristalsis. The same description holds for potentials of the small intestine. Here, epinephrine stops spike activity first, but slow potentials persist as long as there is mechanical activity. Muscle from the intestine of the guinea pig responds only with spikes, and their frequency is related to the magnitude of the contraction. The findings of Kuffler (85) regarding the contractures of frog muscle and their electric manifestations bring the data on visceral muscle closer into line with the better known skeletal muscle, for they show some similarity in behavior.

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DEPARTMENT OF NEUROLOGY  
COLLEGE OF PHYSICIANS AND SURGEONS  
COLUMBIA UNIVERSITY  
NEW YORK, NEW YORK

## ELECTRICAL ACTIVITY OF THE BRAIN<sup>1</sup>

BY MARGARET A. LENNOX AND WILLIAM G. LENNOX

*Yale School of Medicine, New Haven, Connecticut and Department of Neurology,  
Harvard University Medical School, Boston, Massachusetts*

The electrical activity of the brain may be discussed at three levels. First is the origin and the nature of the nerve impulse. This most important subject involves understanding of the physico-chemical processes of the individual neuron—problems of cellular respiration and metabolism, interaction of electrolytes, permeability of membranes, and concentrations of ions—and the means by which the manufactured electrical energy traverses the fiber. Progress in this area of knowledge is slow. Bronk (1) gives a partial, but thought-provoking review. Second is an explanation of the mechanisms which lie between the energy of individual cells and the recordings of the electroencephalograph, the means by which the impulses from billions of individual neurons are integrated, and the meaning of the various wave forms which are traced by the oscillating potentials of the cortex. Third is the insight into the normal and abnormal functioning of the brain which a study of these oscillations gives. This review deals with the last two levels only.

Direct studies aimed at the clarification of fundamental physiological mechanisms have been disappointingly few in the past two years, the period covered by this review. Portions of the problem which require clarification deal with inherent rhythm, problems of transmission, explanation of wave forms, of their points of origin, and of conditions which modify them.

*Inherent rhythm.*—Fluctuation of electrical rhythm is an almost universal phenomenon, a quality inherent in living matter, and perhaps beyond explanation. Bullock (2) adds his own observations on the electrical activity of the invertebrate nervous system to a review of the work of others. He concludes that the regular rhythm represents synchronized beating of many cell bodies rather than a summation of faster frequencies dependent primarily on nerve impulses.

*Transmission of the nerve impulse.*—Loewi (3) reviews the circumstantial evidence for chemical transmission of impulses in the

<sup>1</sup> This review covers the period from July, 1944 to July, 1946.



central nervous system. He concludes that, though not proven to be liberated during central nervous activity, acetylcholine in adequate doses can increase this activity, and suggests that potassium liberated during impulse propagation may in turn liberate the transmitter. Forster (4), on applying a 5 to 20 per cent solution of acetylcholine to the cortex, observed an immediate depression of its electrical activity followed by high voltage spiking discharges. Spikes from the motor cortex were accompanied by clonic movements of the corresponding muscles. When acetylcholine was applied to the acoustic cortex, spikes were increased in number and in their spread to other regions when evoked by auditory stimuli (5). Application of strychnine to the cortex facilitates the spontaneous spread of spikes (6).

Davenport (7) does not agree with Ashby that carbonic anhydrase activity is indispensable for cellular metabolism and electrical activity. Thiophene-2-sulfonamide given to rabbits in a dosage calculated to inhibit 99.9 per cent of carbonic anhydrase activity does not change the electrical response of the cortex to weak and strong tetanic stimulation, nor does it prevent the appearance of spikes in response to sensory stimulation or to the application of strychnine to the cortex.

*Wave form.*—Study of the factors which may alter wave form has been greatly facilitated by the device described by Brooks & Walter (8). As many as ten different frequencies can be generated simultaneously by a photomechanical device. Amplitude and phase relationships of the various waves can be altered at will. The application of this method to the study of the electroencephalogram has been exhaustively discussed (9). The electroencephalogram may contain frequencies which cannot be distinguished by the eye from the dominant frequency. Wave form is determined primarily by the phase relationships of the component frequencies. A somewhat simpler method for generating physically compounded waves (a maximum of three simultaneous frequencies) has been applied by Cohn (10) to the elucidation of psychomotor-like waves in the human electroencephalogram. The addition of low order harmonics in phase to the fundamental wave emphasizes the downstroke of the fundamental wave. Square and asymmetrical waves result from the addition of second and third harmonics to the fundamental wave, whereas symmetrical triangular wave forms result if the third harmonic only is added. Out-of-phasesness alters

the shape of the waves, as do changes in the relative amplitudes of the waves.

*Origin of normal rhythms.*—The fundamental, constitutional nature of the frequency and voltage of waves is demonstrated by the fact that the electroencephalogram seems to be an hereditary trait. Records were made of 74 normal twin pairs by W. Lennox *et al.* (11). Eighty-five per cent of the monozygotic twins had records which appeared to be identical and 95 per cent of dizygotic twins had dissimilar appearing records.

The frequency of the potentials of the electroencephalogram of "healthy" persons is from eight and one-half to twelve per second. This "alpha" rhythm may be automatic and spontaneous, or the frequency may be dictated by a regional "pacemaker." The rhythm is most distinct over the occipital lobes and is easily altered by stimulation of the optic nerve. Darrow (12) postulates a thalamocorticothalamic feedback circuit as essential for the maintenance of the regular alpha rhythm. This theory is not supported by the findings of Cohn (13) and M. Lennox (14) that a normal regular alpha rhythm may be found in the frontal areas of patients subjected to frontal lobotomy.

Williams & Reynell (15) examined a large number of patients with head injuries who had suppression of alpha activity as the chief or only finding. Careful analysis of these cases supports the thesis that the alpha activity arises in the parietal and occipital areas (roughly areas 18 or 19) and is propagated caudally and rostrally. There is some indication that the propagation may occur by neuronal rather than by simple electrical conduction. Suppression of the normal rhythm over an entire hemisphere follows destruction of brain substance or temporary (reversible) suspension of activity of the propagating center. A destructive lesion anterior or posterior to the propagating center may abolish the alpha rhythm in areas distal to the lesion. Thus the extent and severity of suppression of normal frequencies is determined by the site rather than by the extent and severity of the injury. On the other hand, suppression of abnormal brain frequencies is due to damage of brain tissue; and its position, extent and severity is a direct reflection of the position, extent and severity of the injury. These authors believe that all the reported instances of low voltage foci involving suppression of the alpha activity may be reconciled with their findings.

The alpha activity observed in humans appears to arise in the cortex, but other frequencies may be the reflection of subcortical activity. During sleep and anesthesia, formations which resemble spindles appear in leads from the frontal areas but disappear after lobotomy (14). The frequency is eight to fifteen per second in both humans and cats (16, 17). Morison & Bassett (16) report that the spindles are most prominent in the thalamus of pentobarbitalized cats, and persist in acute preparations deprived of all neocortical connections and (in one animal) after removal of both neocortices and division of both optic nerves and transection of the brain stem. In chronically hemidecorticate cats, spindles are not observed, and in these animals degeneration of the thalamic nuclei, especially centralis lateralis and centralis medialis, is prominent. These findings demonstrate that the spindles arise in the thalamus and do not depend on cortical impulses for their maintenance. Ulett (18) observed that sleep spindles fail to appear on the affected side of dogs with experimental space occupying lesions in the subcortical white matter. According to Murphy & Gellhorn (19), spindles in the electrocorticogram of cats can be abolished by hypothalamic stimulation. Absence of spindles during sleep may have some localizing value in patients with tumors or other lesions which interfere with the afferent cortical fibers. Absence of sleep spindles indicates the affected side.

*Origin of abnormal waves.*—The scientific and clinical meaning of abnormal voltage and wave form is most important. Two questions are involved: first, the cause of the abnormal wave form; and second, its place of origin. Rhythms are abnormal because of altered rate and voltage. Is there excessive discharge of certain cells or a synchronization of discharges? As for the area of the brain disturbed, recordings from the cortex of patients identify the locus of abnormal waves associated with localized cortical lesions. Abnormal frequencies in the electroencephalogram may also be the reflection of subcortical activity. Hursh (20) observed bilateral symmetrical petit mal waves in two patients after almost complete section of the corpus callosum. Presumably petit mal waves have their origin in some subcortical center or centers. Although this assumption is reasonable in view of the findings of Hursh and others, it is difficult to explain why typical petit mal waves are not often seen in patients with subcortical lesions, but may rarely be seen as a focal abnormality presumably indicative of focal cortical lesions. In children with evidence of midbrain le-

sions and akinetic seizures, a two per second slow spike and wave (petit mal variant) is often encountered (21). Unlike the three per second dart and dome of petit mal, this slower pattern is most often localized in one area. In some patients, the alternating dart and dome have differing frequencies, the dart climbing the dome.

Paroxysmal, high voltage slow waves, on the other hand, are not infrequently found in patients with evidence of organic damage to subcortical centers. Cobb (22) describes bilateral high voltage rhythmic one and one half to four per second waves in twelve patients with deep tumors situated in or encroaching on the midline. Rhythmic one and one half to four per second waves occurred unilaterally only in the region of the cortical tumors. In the patients with deep tumors observed by M. Lennox (14), waves with the characteristics described by Cobb occurred unilaterally as well as bilaterally. Faster (four to six per second) rhythmic waves were more apt to occur in patients whose lesions involved the hypothalamus and pons. The shape of these waves is peculiar (serrated, bifid, square, etc.) whether the frequency is one and one half or six per second. Waves with these same characteristics are seen in patients with epilepsy of the psychomotor type. In both instances it seems likely that the slow frequencies represent electrical activity of subcortical centers. The peculiar wave form may be imparted by superimposed normal or harmonic frequencies, presumably arising in the cortex (9, 10).

Hypothalamic stimulation may facilitate cortical electrical activity and excitability. Murphy & Gellhorn (19) stimulated discrete areas in the hypothalamus with increase in the motor response elicited by electrical stimulation of the cortex. Areas which give rise to increased cortical excitability also give evidences of sympathetic autonomic stimulation, but sympathetic stimulation is not a necessary condition for the facilitation of cortical electrical activity. Various pathways by which hypothalamic activity may modify cortical activity are outlined by the strychnine spike method (23). Alumina paste injected into the thalamus of monkeys by Jasper *et al.* (24) results in paroxysmal slow waves which appear synchronously over the cortex.

Walter & Dovey (25) postulate that four to seven per second (or "theta") waves are "characteristic of the resting, immature or isolated parietotemporal cortex." They base this assumption on their findings in twenty-one patients with tumors arising from or spreading into subcortical structures. In all cases four to seven per

second waves in the parietal and temporal areas were detected by automatic frequency analysis. The amount and site of delta activity depended on the extent to which the cortex was involved. Theta activity arising in the parietotemporal regions is also a characteristic of the electroencephalogram of normal children and adults in light sleep. Before their assumption can be accepted unreservedly, the factual foundation should be broadened by experimental and clinical observations but, because most of the lesions in human beings are tumors, demonstrated effects may be due either to interruption of afferent fibers (just which ones may not be known) or to increased, decreased or abnormal activity of subcortical centers due to injury. Irregular slow waves in the region of expanding lesions are easily demonstrated in the electrocorticogram. They are much less apt to occur in the electrocorticogram of patients with deep tumors. Slow waves in the neighborhood of deep expanding lesions have been demonstrated in four cases by Walter & Dovey (26) by the technique of direct electrography. They used a brain needle, properly prepared, as an electrode. Further application of this method should help to elucidate some of the properties of subcortical as contrasted with cortical electrical activity in humans.

#### MODIFYING CONDITIONS

*Electrical stimulation.*—Leão (27) reports a depression of cortical electrical activity as a result of tetanic or mechanical stimulation of the cortex. Depression spreads wave-like in all directions from the point of stimulation and may involve the whole ipsilateral cortex except the area striata. When spread occurs to the opposite hemisphere, it is mediated by the white fibers via the corpus callosum. The mechanism of spread in the ipsilateral cortex has not been established. It does not depend on subcortical structures, nor on any but the outer two cortical layers. Axon to axon spread in the fiber system of layer I of the cortex is possible because the depression will not spread past a superficial cut. On the other hand, vascular changes may be responsible. The spreading depression of electrical activity is regularly accompanied by pial vasodilatation and increased blood flow (28), and spread from the frontal areas may be blocked if cocaine is applied to the great branches of the middle cerebral artery low on the temporal lobe (29).

Other interesting and at present unexplained phenomena which occur in conjunction with the spreading depression have been ob-

served and described in detail by Leão (29). Most interesting is the observation that of all the agents tested, potassium chloride alone is effective in initiating the depression of cortical electrical activity. Acetylcholine (concentration not stated) was ineffective. Forster *et al.* (30), describe spreading depression as a result of the local application of acetylcholine to the cortex. It is not clear whether this phenomenon is similar in all respects to the spreading depression elicited by tetanic and mechanical stimulation and by the local application of potassium chloride described by Leão (29). If the two phenomena are identical, then it may be, as Loewi suggests (3), that potassium, liberated during nerve stimulation, is in its turn responsible for the liberation of acetylcholine.

*Vascular conditions.*—Most investigators agree that circulatory changes in the brain do not alter the electroencephalograph. However, Engel, Ferris & Romano (31) report slow waves in the region of the presumed vasoconstriction which accompanies the scintillating scotomata of migraine. Hyperventilation causes both cerebral vasoconstriction and slowing of brain waves, but according to Gibbs *et al.* (32), the greatest slowing (or build-up) occurs in persons whose cerebral arterioles do not constrict in response to the lowering of the arterial carbon dioxide. Slowing is due to lowered tension of carbon dioxide, not of oxygen. Although Engel *et al.* dispute the latter findings (33), they confirm the opinion that electroencephalographic changes are not closely correlated with vascular changes. In the light of these and other findings, it is difficult to accept the contention of Darrow that the electroencephalogram is regulated by autonomic-vascular changes (34). Darrow states that electroencephalographic slowing during hyperventilation is correlated with an increase in heart rate (35) and with an increase in palmar skin conductance (36). In cats, the slowing of waves which results from hyperventilation is aggravated by atropine and by cutting the left facial nerve, both of which interfere with the parasympathetic control of the vessels of the brain. Physostigmine and mechanical stimulation of the distal cut nerve stumps inhibit the slowing which follows section of the parasympathetic nerve supply. In two animals, pial vasodilatation after stimulation was observed through a cranial window. Simultaneous recordings were made of the electrical potentials of brain and heart and of the diameter of pial blood vessels under different conditions of temperature, with asphyxia, increased tension of carbon dioxide

and after administration of atropine and pilocarpine. Increased frequency of potentials tended to parallel vasodilation, and decreased frequency, to parallel vasoconstriction. In a recent review, Darrow (37) postulates a homeostatic mechanism by which "the activity around ten per second in the central nervous system may cause cerebral vasoconstriction" and "the fast activity favors cerebral vasodilatation." This effect may be mediated by an "initiating pace maker in the intralaminar thalamic nuclei" and "this may be given regularity by a parallel resonating feedback from the cortex to the ventrolateral thalamus." This assumption is based on previously noted findings of Morison and Bassett and ignores their finding that the cortex is not necessary for the regular spindles which appear in recordings from the thalamus. In addition, Darrow postulates an afferent feedback from the cerebral vessels. These findings and theories of the mechanism of cortical electrical activity cannot be reconciled with the findings of other investigators who have found no constant relationship between vascular and electroencephalographic events. Resonating circuits between the cortex and thalamus are not indispensable for the maintenance of a normal electroencephalogram for lobotomy may not affect the latter. The discrepancies of opinion in this field calls for further experimentation.

*Mechanical conditions.*—In order to study the effect on the electroencephalogram of pressure applied to the cortex, Glaser & Sjaardema (38) screwed a lucite rod containing silver wire electrodes into the skulls of rabbits. Increasing pressure of the rod caused first fast and mixed fast and slow activity, then slow waves with fast waves sometimes superimposed, and finally absence of activity. Pressures of cerebrospinal fluid were not recorded. Ulett (18) injected paraffin into the brains of unanesthetized dogs. Subcortical injections caused slow waves of the cortex which lasted for approximately seven days. In human subjects slow waves are also most common with actively expanding lesions. In the dogs spindles which accompany anesthesia failed to appear over the injected cerebrum. This observation supports the conclusion of Morison & Bassett (16) that spindles do not appear in the cortical leads when the afferent pathways to the cortex are interrupted. Ulett was not able to determine whether the slow waves arise as a result of the interruption of afferent pathways, or as the result of apparently reversible changes in cortical neurons not demonstrable by common histological methods. When the paraffin mass was injected



subdurally or extradurally only decreased voltage on the affected side resulted, and persisted when the mass was removed. Possibly the explanation for lowered voltage in these animals and in the patients of Williams & Reynell (15) was the same. As previously mentioned, mechanical pressure may cause a spreading cortical depression.

*Metabolic conditions.*—The relationship of the electrical activity of the brain to its oxygen tension is most important. Davis, McCulloch & Roseman (39) used unanesthetized cats; they were immobilized with curare and maintained with artificial respiration. Convulsions were induced electrically and by intravenous injection of amino acid, caffeine, coramine, metrazol, strychnine or picrotoxin. Several seconds before the electroencephalographic change which heralded the convulsion, there was an abrupt drop in the oxygen tension of the cortex which persisted until about ten seconds after the last clonic jerk. The drop was independent of any blood pressure change and is interpreted as due to increased cerebral metabolism. From chemical analysis of brains obtained from convulsive dogs, Stone *et al.* (40) conclude that there is greatly increased brain metabolism during convulsive activity and that a relative anoxemia develops.

*Blood gases.*—Inhalation of nitrogen by the curarized cats of Roseman *et al.* (41) caused a prompt fall in the oxygen tension of the cortex associated with a decrease and final disappearance of electrical activity. A 75 per cent increase in oxygen tension produced no electroencephalographic changes, but when convulsions were produced by very high oxygen pressures, the electroencephalographic changes resembled those induced by other measures (42). Gurdjian *et al.* (43) studied cerebral metabolism in hypoxia in dogs. When the oxygen saturation of arterial blood was less than 55 to 65 per cent, the concentration of the lactic acid of the brain increased, and the brain waves began to slow, the slowing increasing progressively with the anoxemia. A close correlation between electroencephalographic slowing, rise in lactic acid, or fall in carbon dioxide was not established in these few studies. Decomposition of phosphocreatine occurred when the oxygen saturation of arterial blood fell from 25 to 35 per cent.

Early electroencephalographic studies demonstrated the slowing of cortical potentials which accompanies overventilation. The degree of slowing, various authors agree (44, 45, 46), is modified by

the glucose level of the blood, increased slowing going with low values and decreased slowing with high values. Gross changes in brain wave frequencies follow gross changes in the pH, or in the glucose, oxygen, or carbon dioxide concentration of the blood passing through the brain. Minor changes in the concentration of these chemicals do not, according to Engel *et al.* (33), show consistent correlation with electroencephalographic changes. In some individuals changes are consistent with the assumption that hyperventilation produces cerebral vasoconstriction, in others that it causes cerebral vasodilatation. Neither group shows consistently correlated electroencephalographic changes.

*Water balance.*—Acute water intoxication of rats results in high voltage one to three per second waves combined with single or multiple spikes, according to Gellhorn & Ballin (47), but Prados, Strowger & Feindel (48) found that cerebral edema produced by operative exposure of the brain, and causing increased capillary permeability and swelling of the cells, resulted in lowered voltage waves, with both irregular delta and fast activity. During the period of recovery, high voltage spiky waves are mixed with irregular slow waves. Both histological and electroencephalographic changes were prevented by the preoperative administration of the extract of the adrenal cortex. Frogs immersed in distilled water lost a calculated 40 per cent of their chloride and exhibited waves which were slow, from two to eight per second, and of very low voltage. These changes could be reversed by the injection of sodium chloride, potassium chloride or vitamin B<sub>1</sub>. The authors, Pick & Miller (49), did not determine definitely if the changes were due to the loss of chloride or to the loss of cations or anions as well. Blood from the superior sagittal sinus of dogs subjected to electric shock or chemically-induced convulsions contains an increased concentration of potassium, according to Cicardo (50).

*Hormones.*—Odoriz *et al.* (51) observed increased beta activity in the electroencephalograms of seventeen patients with parathyroid insufficiency, and regular alpha activity in compensated cases. There was no close correlation between the electroencephalogram and blood calcium level. Taubenhaus & Engle (52) observed regular alpha activity in a single patient whose blood calcium was abnormally low. Beta activity predominated when the blood calcium reached a normal level. Cress & Greenblatt (53) noted no alteration of the electroencephalogram after the administration of stilbesterol

and progesterone, alone and in combination, in three postmenopausal women.

*Drugs.*—Sedative drugs (54), barbiturates (55), and bromides, (56), in small doses, cause an increase in the amount of 20 to 25 per second activity chiefly in the frontal areas, while in larger doses they cause slowing. Similar changes occur in infants under pentothal anesthesia (57). Penicillin and allied substances may cause electroencephalographic abnormalities when injected intravenously (58), or when given intrathecally or applied to the cortex (59). Subconvulsive doses of metrazol produce slow waves or spike and dome discharges which are antagonized by trimethyloxazolidine dione (tridione). In cats tridione protects from metrazol better than do barbiturates. These authors summarize clinical and electroencephalographic evidences of the anticonvulsant effect of dilantin in animals (60). In patients, trimethyloxazolidine dione has a curiously selective action in eliminating the alternate three-per-second dart-and-dome formations of the electroencephalogram and the associated petit mal triad of epileptic seizures (21). In dogs, *d*-tubocurarine produces first stimulation and then depression of cortical electrical activity (61). Chronic convulsions appear in monkeys from three to eight weeks after local application of alumina cream to the motor cortex (62). Electroencephalograms obtained under sodium pentobarbital anesthesia contain persistent focal fast and slow activity not found in the control animals. The lesions are similar to meningocerebral cicatrices in human beings (63).

#### CLINICAL OBSERVATIONS

Reviews of clinical electroencephalography have been written recently by Gibbs (64), Williams (65), and Kornmüller (66).

*Epilepsy and related states.*—Because patients with epilepsy most frequently display cortical dysrhythmia, and because the brain-wave pattern is a hereditary trait, electroencephalographic studies of epileptic twins and of relatives of patients help in judging the relative etiological importance of heredity and of brain damage (67).

Echlin (68) prefers the Jasper to the Gibbs classification in interpreting the records of patients with Jacksonian seizures. Roseman (69) classified the records of 364 epileptics encountered in the Army and found the distribution similar to that of Gibbs' civilian patients.

Convulsions which occur during anesthetization have long been a puzzle. Fifteen out of twenty-two persons with such a history examined by Williams & Sweet (70) had abnormal electroencephalograms, indicating a factor of predisposition. On electroencephalographic evidence, Cohn & Cruvant (71) postulate a fundamental relationship between narcolepsy and epilepsy. Their illustrative records are far from convincing. Patients with tumor of the islet cells of the pancreas and with convulsive states and coma have much electroencephalographic abnormality (72). Of diabetic patients with a history of repeated insulin reactions 51 per cent have abnormal electroencephalograms (73).

Dysrhythmia accompanies the impairment of consciousness of carotid sinus syncope (74). In two patients having the cerebral type, focal slow waves appeared on the side which was pressed upon. On the other hand, neither Roseman *et al.* (75) nor Rogers (76) observed focal abnormalities after ligation of an internal carotid artery. Persons with syncope, either "spontaneous" (77) or induced by anoxemia (78), do not display any dysrhythmia. Methods (aside from deep breathing) of evolving latent high voltage "seizure-discharges," are important; sleep uncovers a surprising number (79) with electrodes on the patient's cortex; a minimal electrical stimulation will identify an epileptogenic focus (80).

*Tumors.*—Aird & Bowditch (81) claim more accurate localization by a quantitative (and time-consuming) expression of focal electroencephalographic changes than is possible by scanning the record. Changes in wave frequency and form are thought to give some indication of the nature and degree of malignancy of brain tumors (82, 83). Facetious patients with organic brain disease tend to have more delta activity in the frontal area than other patients (84).

*Head injury.*—According to Roseman (85), the appearance of focal or generalized fast activity after penetrating wounds of the brain is a grave prognostic sign, justifying the prophylactic administration of diphenyl hydantoin. Mild concussion, on the other hand, causes little dysrhythmia even within thirty minutes after the head injury (86). Three months or more after trauma to the head, even though headache and dizziness persist, electroencephalograms are almost uniformly normal (87). Irregularities in rhythm, except for larval epileptic waves, which almost certainly presage epilepsy, are of little value in the diagnosis of post traumatic epilepsy (88).

*Organic psychoses.*—In delirious patients, the appearance of the electroencephalogram could be correlated with the degree of impaired consciousness (89, 90). In neurosyphilis, abnormalities are more common with cortical involvement (91); when evoked during fever therapy are apparently due to hyperventilation (92), and are usually reduced by penicillin therapy (93). Normal electroencephalograms are common in patients with alcoholism (94), delirium tremens (95), arteriosclerosis and senility (96). The waves in acute encephalitis may be indistinguishable from those in psychomotor epilepsy (97).

*Functional central nervous system diseases.*—In an excellent review, Hill (98) emphasizes that the electroencephalogram is much less valuable in functional than in organic mental diseases. Earlier reports of abnormalities in psychopathic individuals did not take into account the changes with age, with low blood sugar, or with medication. Electroencephalograms of 452 criminals did not differ greatly, age and other factors considered, from those of 1,432 "controls" [Gibbs *et al.*, (99)]. However, reports of electroencephalographic abnormalities in personality disorders persist (100 to 105).

Homosexuals in a criminal population, according to Silverman & Rosanoff (106), have histories suggesting much structural abnormality of the brain and much dysrhythmia. Ostow & Ostow (107) consider a high incidence of high-voltage slow waves in criminals, which appear only during or after hyperventilation, important, although this is a doubtful inference. Certain variations in the type and onset of sleep patterns are considered significant for different forms of psychosis (108). No electroencephalographic changes occur during hypnosis (109). Obviously, the "normal" hyperventilation and sleep patterns require clearer definition. The same may be said for the electroencephalograms of children, though Henry (110) has performed a public service through his monograph.

*Problem children.*—Gottlieb *et al.* (111) find electroencephalographic abnormality in many problem children, most of whom have a history of complicating factors. Electroencephalography was of no help in the study of other children (112, 113). The review by Will (114) illustrates the difficulties involved. Altogether, no very definite conclusions can be drawn from these scattered studies which attempt to clarify aberrations of thought and action. Elements in the uncertainty are: the vague boundaries of psychosis and abnormal behavior; the smallness of the groups studied which

does not permit subdivision into the various types and degrees of dysrhythmia; differences in apparatus, technique, and interpretations; and the absence of adequate control observations. No studies in the fields of abnormal thought or actions compare with the classification of the electroencephalograms of 1,000 controls and 1,260 epileptics made by the Gibbises (115).

*Electric shock therapy.*—Changes in cortical electrical activity, which take place during and after electric shock convulsions, are reviewed by Pacella (116). Only a few of the patients who showed pretreatment dysrhythmia have since developed spontaneous convulsions (117). Differences in type of current used and in electrode placement influence the degree of postshock slowing of brain waves both in patients (118) and in animals (119).

Electronarcosis produces slowing of waves similar to that induced by electric shock. This slowing is reduced by thiamin and niacin (120). Postconvulsive-slowing in monkeys is eliminated by amphetamine alone of the drugs tested (121). The anticonvulsive and antidysrhythmic effect of drugs in animals subjected to electroshock has been studied (122).

#### INSTRUMENTATION

The frequency of the electrical potentials of the brain is of prime importance in the interpretation and classification of records, yet a method of counting has not been agreed upon. Engel *et al.* (123) count the number of waves in each second of a 300-second strip, whereas Brazier & Finesinger (124) determine the incidence of the waves of various frequencies. Kaufman & Hoagland (125) point out the advantages of the latter method. Automatic frequency analysis, used first by Gibbs in this country, is now employed in England with use of the method devised by Brooks & Walter (8). The analysis is performed for each ten-second interval and is traced over the primary record. The height of the deflection at each frequency from 2 to 22 cycles per second represents both amplitude and duration.

"Thus, a 10 cycle per second oscillation with an average amplitude during the epoch of one half the possible maximum lasting throughout the epoch would be represented as a kick of the analyzer . . . of the same size as an oscillation of maximum possible size but lasting for only one half the epoch."

A different device for continuous frequency analysis of the electroencephalograms was described by Lowenbach & Barbour (126). Recording by means of a brain needle from deep brain tissue was

described by Walter & Dovey (25). Using monkeys, M. Lennox & Ruch (127) placed electrodes produced by the injection of alumina cream in the ventricles and lateralized deep brain lesions and compared the wave forms with those from scalp electrodes. Recommendations for the design and performance of the electroencephalographic apparatus are outlined by Dawson & Walter (128). Finally, Donovan (129) reminds us of the variety of services which electronics is giving and can give to medicine and to neurology.

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YALE SCHOOL OF MEDICINE  
NEW HAVEN, CONNECTICUT  
HARVARD SCHOOL OF MEDICINE  
BOSTON MASSACHUSETTS

## THE SOMATIC FUNCTIONS OF THE CENTRAL NERVOUS SYSTEM

BY CLINTON N. WOOLSEY

*Department of Physiology, School of Medicine, Johns Hopkins University,  
Baltimore, Maryland*

In the following review of contributions published mainly during the year ending July 1946 effort has been made to relate the results in certain fields to earlier studies, to comment critically on the findings and to suggest in some instances questions for further inquiry. If the story appears somewhat unbalanced it is because the author feels that opinions and judgments can be of value only if they are based on experimental contact with the questions discussed. This accounts for the emphasis placed on cerebral cortex and cerebellum.

### CEREBRAL CORTEX

*Somatic afferent areas.*—Study of localization in somatic afferent areas with the aid of vacuum tube amplifiers has been continued. It has been established in a variety of mammals that, in contrast to what has been held generally heretofore, each cerebral hemisphere has two somatic receiving areas (1 to 7). These two are somewhat differently organized and each retains its own basic characteristics in the various species studied (7). Duality, however, is not limited to the cutaneous afferent system; it is also present in the visual (8, 9) and in the auditory (10, 11). Duality, therefore, appears to be a fundamental principle of organization in the cortical afferent centers. In fact, since there is evidence that the cerebellar receiving areas also are double (see section on the cerebellum), it seems that a dual system of organization may pervade the afferent nervous system. One is reminded of the duplicity theory of vision (12), of spiral and radial fibers in the cochlea, and of the evidence, recently reviewed by Bishop (13), for two types of fibers for pain and certain other modalities in peripheral nerve. One wonders indeed whether, after all, there may not be an anatomical and physiological basis for Head's postulated dual mechanisms of cutaneous sensibility! In the cerebellum the dual system involves also the effector apparatus. In the cerebral cortex, the second somatic area appears to coincide with one of the extrapyramidal motor fields (14). This raises a question for future study: what are

the relationships of the dual afferent systems to the cerebral motor mechanisms?

It has been suggested (7) that the dual cortical afferent systems be referred to as somatic areas I and II, visual areas I and II, and auditory areas I and II. This terminology carries no anatomical or functional implications and it can be applied to each of the three systems. Moreover, area I corresponds to what has been considered generally the primary receptive area of each system; area II in each case was "second" in time of discovery.

Somatic area I is the postcentral gyrus of the monkey (Brodmann's areas 3, 1 and 2) and its homologue in other animals. This area is basically the same from species to species, but differs in the relative development of its various subdivisions, apparently in accord with the degree of specialization of the corresponding peripheral receptor surfaces in individual species. Except for a portion of the face area, in which the relationship to the periphery is ipsilateral, somatic area I has been found to be strictly contralateral in its connections. The pattern of localization within this area is very detailed (15), so that each cortical point is optimally related to some particular portion of the cutaneous surface, giving a kind of point-to-point projection. At the same time there is considerable overlapping of the representations of the various portions of the cutaneous surface. Doubtless, these two features of cortical organization are important respectively for the functions of localization and of integration.

Somatic area II lies mainly on the dorsal wall of the sylvian fissure in the monkey; in other species (rabbit, cat, dog, pig and sheep) it is situated in a homologous region lateral to somatic area I and rostral to the auditory cortex. Somatic area II was first described by Adrian (1, 2) as a "second" somatic receiving area in the cat. He reported that it received impulses from the contralateral claws and ventral side of the toes. It has since been found also in the dog (3), monkey (3, 4, 7), rabbit (5), pig and sheep (7). In all species, including the cat (7), somatic area II of each hemisphere is related to the entire cutaneous surface of both sides of the body. Responses evoked by mechanical stimulation of the contralateral half of the body are about twice as large as responses produced by stimulation of corresponding points on the ipsilateral surface. Apical parts (snout, digits, tail) give largest responses on stimulation, and under deep barbiturate anesthesia the apices of the contralateral half of the body alone may activate the cortex.

These results were first clearly demonstrated in the rabbit (5) and have since been confirmed in most details for the other animals cited. In contrast with somatic area I, where relative richness of peripheral innervation is reflected in extent of cortex devoted to a part, in somatic area II it is the amplitude of response which appears to vary directly with the density of peripheral innervation. In somatic II there is much greater convergence of pathways on the cortical field and point-to-point projection is less obvious. Nevertheless, there is spatial differentiation in the projections of face, arm, and leg, and this increases definitely from the rabbit, where the respective foci of maximal response are approximately one mm. apart, to the monkey, where face, arm, and leg subdivisions are relatively well differentiated and overlap each other less extensively.

In the rabbit (5) it has been shown that somatic areas I and II have separate projection pathways to the cortex. Latency measurements and the fact that either may be suppressed by the anesthetic, depending on the animal, without suppression of responses in the other area, also indicate that the two areas have separate pathways into the cortex. Therefore, whatever may prove to be the functional interrelations of the two systems, area II clearly does not stand in a secondary relation to area I as regards the pathways from the thalamus, and it ought not to be referred to as a secondary somatic sensory area.

It has been suggested (7) that somatic area II may be more ancient phylogenetically than area I because of its less obvious intra-areal differentiation and because of its position relative to somatic area I and the older rhinencephalon. This possibility seems less likely at present. Preliminary studies indicate that somatic area II is interrelated bilaterally with both paramedian lobules of the cerebellum. These lobules, as discussed in the section on the cerebellum, appear to have undergone more extensive development later in phylogeny than have the cerebellar areas related to the Rolandic cortex. This suggests that somatic area II may reach its highest development in the higher primates. Thus, it may have to do with some of the so-called higher functions of the brain, possibly functions which have been attributed to association areas. If this be so, the concept of association areas will have to incorporate the facts detailed above. However, the writer doubts that areas II will prove to be association areas.

In this connection Allen's (16, 17) results are of interest. In a

conditioned reflex study on dogs, he found that bilateral ablations of his area C (somatic area II) disrupted completely the association mechanism for making correct conditioned differential responses with two sets of general cutaneous stimuli, although positive conditioned responses not involving differentiation were unaltered. Reestablishment of the differential response required 839 to 987 trials when the conditioning stimuli consisted of stroking the back "with and against the grain" and a lesser number, later, for slow and rapid strokes. More extensive bilateral lesions, extending into his area B (auditory area II), prevented formation of new association circuits capable of producing correct responses with both sets of cutaneous stimuli during a very long and intensive period of testing.

Other studies which are of possible interest in relation to somatic area II are those of Bender (18, 19) who found (18) in certain war injury cases dulling or extinction of cutaneous sensation in the affected area when this and a normal area, usually the corresponding one of the opposite side, were stimulated simultaneously. When the affected area alone was stimulated it might be fairly sentient. In other cases pain was precipitated, or spontaneous pain aggravated in a causalgic limb when the opposite hand or foot or some distant part of the body was stimulated. The author hypothesized that both of these phenomena might be explained by bilateral cortical representation. Such a bilateral representation exists in somatic area II.

It was noted above that part of the face subdivision of somatic area I has ipsilateral relations with the cutaneous surface. This ipsilateral area has been identified in the rostral portion of the face area in the rabbit, cat, dog, pig, sheep, and monkey (7). It is separate and distinct from the bilateral face representation in somatic area II. The ipsilateral area was first encountered by Adrian (20) in a study of ungulates, in which it was found that only receptors in the lips and snout sent impulses to the face area of the cortex. In sheep and goat the impulses were ipsilateral while in pig and horse they were contralateral. Restudy of sheep and pig (7) revealed that ipsilateral and contralateral systems were present in both animals. Further study showed that the ipsilateral area is present also in the rabbit, cat, dog, and monkey (7). In all species the same portions of the face are concerned, i.e., lips, lower side of face, tongue, and buccal cavity. The area varies in size from species to species. Its significance is as yet uncertain (20, 21).



Further observations on the somatic receiving area of the Shetland pony have been made (21) and a more detailed map of the limb area has been secured. "The receiving area is made up of an anterior part in which the contralateral nostril is represented in considerable detail and a posterolateral part of about the same size for the contralateral half of the body and the fore and hind limb." It seems probable that the posterolateral part, lying as it does near the auditory area (20) and showing marked convergence of afferent pathways, belongs to somatic area II, whereas the anterior part with its detailed representation of the nostril may be a portion of somatic area I. These opinions are supported by comparison of the fissuration of the horse, pig, and sheep brains. That the lateral area is not the only one for the limbs in the ungulate brain has just been shown. Lack of responses from both sides of the body in the limb area could be due to the anesthetic (7).

From oscillographic studies a clearer conception of cortical homologies in brains of diverse morphology becomes possible. Thus somatic area II (Allen's area C in the dog) cannot be a portion of the temporal lobe (16) and the regions ablated by Gobbel & Liles (22) are only in part homologous with parietal cortex of primates. Garol's map (23) of the cat also is obviously incorrect in several major respects. Moreover, if one may judge from the arrangement of the tactile areas in the rabbit, it is unlikely that the areas identified as 3, 1 and 2 in the rat by Krieg (24) are the homologues of the same areas in the monkey, since the areas do not extend throughout the face, arm, and leg subdivisions as they do in monkey.

The war has provided one very interesting study of the effects of localized injury of the human brain (25). In contrast with the instantaneous, widespread arrest of brain activity and the slow recovery generally seen in civilian cases of head trauma, with injuries caused by high velocity missiles the effects of local brain damage usually were found to dominate the clinical picture while the rest of the brain was often so little injured that consciousness was not even temporarily impaired. At time of wounding there was occasionally momentary stimulation, but usually there was immediate extinction of local activity as evidenced by numbness and paralysis. In general, sensory and motor defects occurred together initially. We shall discuss the sensory disturbances here and refer to the motor effects later.

It was observed that when part of the arm area was injured

the symptoms, soon after wounding, involved the whole upper extremity to the shoulder. While the effects often spread to the upper trunk and the face, the lower limb was seldom involved. The same was true for the leg. Attention was called to the similarity of these effects to the action of strychnine in activating a whole face, arm, or leg subdivision. A similar tendency for the pathological disturbance in sensory epilepsy to be restricted to one or two of the major subdivisions defined by local strychninization has been pointed out (26) in discussion of Sittig's cases (27).

The sensory disturbances which followed local injury of the parietal lobe consisted of early profound loss of all forms of sensation in one of the opposite extremities. In the first days or weeks after injury widespread anesthetics shrank to smaller localized areas showing segmental or peripheral distribution with loss of all forms of sensation. Pain, temperature, touch, and vibration senses usually recovered later while some loss of postural and stereognostic senses remained. In some cases, however, profound impairment of all forms of sensation persisted, most frequently after severe injury of a part of the postcentral area close to the central fissure. With more superficial wounds situated more posteriorly in the upper parietal lobe, higher sensory discriminative functions were impaired without loss of primary forms of cutaneous sensibility. The author remarks that the chief difficulty in interpreting the effects of these small lesions of the sensory cortex lies in the fact that in gross wounds of the same area, destroying most of both the motor and sensory cortex with production of hemiplegia and loss of discriminative sensory functions, the patient usually recovered good cutaneous sensibility in the affected limbs.

Finally, attention should be called to an excellent review by Clark (28) of literature (1938 to 1942) dealing mainly with afferent systems. A comment may be made in connection with his discussion of a clinical case of porencephaly in which there was almost complete hemidecortication with practically total loss of all dorsal thalamic nuclei. Only the centre median nucleus and a narrow subependymal zone of cells lining the third ventricle—the latter possibly paraventricular complex of the epithalamus (29)—remained unaffected. Comparison of these findings with the less complete thalamic atrophy generally described after decortication in lower mammals suggested to Clark "a progressive 'corticalization' of thalamic functions which reaches its acme in the human brain."

However, it is probable that the difference between the behavior of the thalamus of lower mammals and of man is more apparent than real. There is suggestive evidence (30) that the whole dorsal thalamus of rabbit will degenerate after removal of all cortex, if the olfactory cortex is included. It seems likely, then, that the apparent difference should be attributed to incomplete decortication in experimental material. The study of Morison & Bassett (31) on spontaneous electrical activity of the thalamus in cats after extensive cortical ablations should be considered in this connection.

Attention may be directed here to a paper (32) on the nuclear configuration and cortical connections of the human thalamus and to two (33, 34) on thalamic degeneration after frontal lobe damage.

*Taste.*—Studies on the localization of the cortical area for taste have been continued. Following a suggestion derived from the earlier finding that lesions of N. ventralis posterolateralis (arcuate) of the thalamus produced marked impairment of taste, the inferior end of the somatosensory cortex, to which the nucleus projects, was ablated in four monkeys and one chimpanzee, and taste function was tested by the preference method before and after the lesions (35). Taste deficits occurred only when the buried cortex of the frontoparietal operculum was invaded, but even with such lesions the disturbance was transitory. More serious, and possibly permanent, loss of taste occurred when the deep opercular cortex was ablated (36). These studies led to the conclusion that taste is localized neither in the traditional hippocampal area nor on the free cortex of the operculum but in the parainsular area.

In an earlier study on the cortical and thalamic centers for taste in the rabbit, Gerebtzoff (37) found that the insular and adjacent cortex gave rise to characteristic augmentation of spontaneous electrical activity when gustatory stimulations were applied. Retrograde thalamic degeneration suggested that the gustatory pathway terminated in the arcuate nucleus and then relayed to the insular cortex. Unfortunately, the lesions were not limited to the insular cortex and so the thalamocortical relation cannot be considered conclusively established. However, the possibility that taste is represented in the insula must be considered in interpreting the experiments of Ruch & Patton (36), for the most effective lesion was so close to the insula that it may well have interrupted thalamoinsular fibers. In any case it is clear that we are near to knowing the locus of the cortical center for taste.

*Visual areas.*—There is relatively little new work on the visual cortex. However, attention is called to two important surveys (38, 39) dealing with the visual system, which appeared some time ago but have not been noted in these reviews.

A recent Ferrier Lecture (40) was devoted to discussion of the organization of the human visual cortex. The conclusions were drawn that

"in man primary visual perception, including color vision, relative localizations in space and perception of form, is subserved by the cortex of the striate area, and that though there is an exact geometric or point-to-point projection of the retina on this area its functional organization is not rigidly determined by this point-to-point representation, but is to some extent plastic and modifiable. More highly differentiated visual functions, which are developed by the association of visual with other sensory impressions, on the other hand, depend on the integrity of the brain outside the striate area."

The occurrence of polyopia and monocular diplopia after cerebral injuries was studied on war casualties (41). A paper on the macular portion of the optic radiation (42) claims to support the view that double representation of the macula involves corpus callosal fibers, but the evidence is inconclusive.

Projection of the visual fields on the cerebral cortex of the rabbit has been studied oscillographically using the evoked potential technic (9). It was found that the region responsive to photic stimulation includes Rose's areas striata, parastriata, and occipitalis. Within this region the contralateral visual field is twice represented, as was earlier shown for the cat (8), in such a way that the two projections are roughly mirror images of one another. The line of division between visual area I, situated posteromedially and visual area II, lying anterolaterally, is a straight line running at an angle of approximately  $45^\circ$  to the sagittal plane. This line corresponds to the vertical meridian through gaze, which in the visual field is located about  $22^\circ$  from the sagittal plane rostrally. The nasal  $22^\circ$  of field, then, project to the ipsilateral cortex on either side of the line separating visual areas I and II, thus forming with the corresponding portion of the contralateral projection a binocular band about 2.5 mm. wide in each visual area. The remainder of the visual cortex in areas I and II is devoted to monocular vision of the contralateral eye. The peripheral field was found to be represented at least out to  $150^\circ$  in area I and to  $100^\circ$  in area II. The extent of peripheral field represented in area II contrasts

with the earlier findings in the cat (8). The horizontal meridian projects close to the posterolateral border of the visual cortex in both I and II except for a strong rostromedial dip about the vertical meridian line, through central vision. Consequently, the upper visual field has small representation except here. The lower field below  $15^\circ$  is likewise compressed.

The vertical meridian on the cortex as defined by these experiments is rotated approximately  $90^\circ$  to the orientation described by Putnam & Putnam (43) and the ipsilateral projection is less extensive than that suggested by O'Leary & Bishop (44) for this animal.

Visual area II in the cat has been homologized with Brodmann's area 18 of monkey (8). The fact that visual area II is entirely anterolateral to visual area I suggests that area 18 in rat has been incorrectly identified (24) and raises a question as to whether 18 completely surrounds 17 in monkey as indicated by McCulloch (45). The great enlargement of the area of cortex subserving central vision in area 17 of monkey makes it appear that 18 completely surrounds 17. However, there should be at least a small region of direct contact between area 17 and the retrosplenial areas on the mesial surface. This point is important when considering the basic plan of cortical organization.

*Auditory areas.*—Ades & Felder (46) made a comparative study of the cortical auditory areas of monkey, cat and rat. In monkey and cat they recorded the cortical potentials evoked by click stimulation before and after placing lesions in the medial geniculate body, brachium of the posterior corpus quadrigeminum, or lateral lemniscus. The boundaries between postoperative active and inactive areas were sharp in monkey, but in cat there was always an intermediate zone of impaired function. No auditory responses at all could be detected in rat. The authors take these results to imply increasing specificity of point-to-point relations from rat to monkey and cite as supporting evidence studies on retrograde degeneration. Since in our experience pentobarbital may affect quite variously different afferent systems in the same animal and the same system in different animals, failure to record auditory responses in the rat could have been an artefact of the anesthesia.

Raab (47) concludes from a study on cats that

"auditory conditioned responses are normally mediated cortically by an all-or-none mechanism of the auditory cortex. In the absence of the projection areas, these

habits may be relearned at a midbrain level and are mediated by the inferior colliculi (discharging through the superior colliculi) with no loss of acuity. After destruction of these cortical and midbrain centers, conditioned responses may still be mediated by bulbar auditory nuclei, although threshold measurements reveal a marked impairment of sensitivity."

Position of the auditory area has been illustrated for ungulates 20, 7), the rabbit (7), and the monkey (7). In the latter, auditory areas I and II were defined.

Discovery that the cochlea is represented more than once in each hemisphere (10, 11, 48) and study of corticocortical relations with the strychnine method (49, 50) have resulted in an understandable confusion (51, 52) in the terminology of the auditory cortex. When dual representation of the cochlea was first described for the cat (10) the old term "primary auditory area" was retained for the low threshold field and the high threshold region was described as a "second auditory area"—after Adrian's "second" somatic (1). Ades (50) by applying strychnine to the "primary" auditory field found that spikes appeared in the posterior ectosylvian gyrus in a region overlapping the basal end of the "second" area. He considered this a "secondary" auditory cortex in the sense of an association area. Tunturi (11) in the dog confirmed the dual system described for the cat and suggested that the areas be termed "dorsal" and "ventral" to avoid functional and relational implications. These terms unfortunately lose their appropriateness in monkey where the two areas are no longer dorsal and ventral (7). More recently Tunturi (48) has described a third area, which creates a further problem, since it lies in the midst of somatic area II. Allen (53) refers to the three areas as A, B, and C. Bailey *et al.* (49) in monkey and chimpanzee found that the "primary" auditory cortex (area 42) "fired" into area 22 and suggested that 22 bore the same relation to 42 as that existing between areas 17 and 18 of the visual system. McCulloch (45) refers to areas 42 and 22 as "primary" and "secondary" auditory centers. These do not correspond to our auditory areas I and II in the monkey (7), since area II is on the insular side of area I, which is McCulloch's "primary" auditory area. If, as we think, auditory areas I and II are analogues of visual areas I and II (areas 17 and 18), then the relation of area 22 to the "primary" auditory area cannot be similar to that between striate and parastriate areas.

It would appear from these various studies that not only is

there more than one auditory receiving area (10, 11, 48) but that there are probably intracortical connections between these areas (50), and additional connections with surrounding cortex (45, 49). These possibilities must be kept in mind in descriptions of the cortical auditory system.

*Cingular and adjacent areas.*—The mesial surfaces of the hemispheres no longer escape experimental study. For some time it has appeared likely that the gyrus cinguli may have to do with visceral functions. Evidence to this effect has been secured in monkey by Smith who concludes from stimulation (54) and ablation (55) experiments that Brodmann's area 24 is a motor field for autonomic and emotional activity. But this area is also one of the suppressor areas revealed by strychnine (56) and its excitation produces profound inhibitory effects on skeletal muscle (14, 54, 56).

Based on the idea that the highly granular retrosplenial areas might constitute a visceral afferent region related to autonomic motor centers in rostral cingular cortex, the mammillary body was stimulated electrically and the potential changes evoked in the ipsilateral gyrus cinguli were recorded (57). In cats two cortical areas of response were found. One lies above the posterior half of the corpus callosum and extends around the splenium posteriorly and into the visual area superiorly. Often this area alone was activated and it always gave largest responses with shortest latencies. The second area is on the mesial surface of the frontal lobe below the level of the corpus callosum. Here records showed an early small surface positive wave and a later slower positive deflection. Evidence suggested that the retrosplenial area might relay to the rostral area. In monkey only areas 23 and 29 were activated.

Autonomic centers have often been reported in the dorsolateral portions of the frontal lobes (58) but whether, and in what way, these may be related to the gyrus cinguli has yet to be worked out. The connections of the cingular cortex with the outer aspect of the hemisphere have been studied with the strychnine technic (56). Perhaps a bridge between the two is now under construction; for two studies have been made of cortical autonomic control for the eyes which involve mesial and dorsolateral aspects of the frontal lobe. In monkey (59) an area for pupillary dilatation extends from sulcus cinguli to upper end of inferior precentral sulcus, near which its focus lies. In the cat (60) the homologous area is chiefly



on the mesial surface above the level of the corpus callosum and rostral to the cruciate sulcus. Electrical stimulation of the focal area in cat caused wide dilation of pupils, complete retraction of nictitating membranes, and marked exophthalmos. In both animals the mechanism of dilatation was found to involve sympathetic excitation and parasympathetic inhibition, rather than the latter alone as has been reported previously.

Murphy & Gellhorn (61) used strychnine to study diencephalic-cortical relations in cat and reported that anterior and posterior hypothalamic nuclei (including the mammillary bodies) discharge to the dorsomedial thalamic nucleus. The anterior nuclei, which project to the gyrus cinguli, apparently were not examined. Additional strychnine and passage of time led to spiking in ipsilateral and contralateral motor and other cortical areas. Strychninization of the cortex revealed correspondingly widespread connections with the hypothalamus. Many other details were reported. These authors also studied (62) the effects of electrical stimulation of the hypothalamus on the movements produced by motor cortex excitation and interpreted their findings to indicate facilitation of cortex by the hypothalamus. On the other hand, Rhines & Magoun (63), after similar experiments, concluded that diencephalic stimulation facilitates cortically induced movements not at the cortex but within the spinal cord.

*Motor cortex: stimulation.*—The manner in which the skeletal muscular system is represented in the precentral cortex continues to be a subject of experiment and discussion. Murphy & Gellhorn (64) in a paper on "multiplicity of representation versus punctate localization" have undertaken "to shift emphasis from isolated cortical representation to more inclusive cortical function." To this end they used suprathreshold electrical stimulation in the rabbit, cat, and monkey and endeavored "to evoke as much as possible rather than as little as possible" and then to determine whether or not the results were due to purely local activity. The intensity of muscular response and the temporal sequence of participation of the various muscles contributing to a movement were not noted because they did not believe "such observations to be relevant to the particular problem under study." They concluded that movements of the various joints of a limb or parts of the face are practically coextensively represented and that there is also considerable overlap between leg and arm, and arm and face subdivi-

sions. Multiplicity of representation was described as characterizing isolated cortical foci also. This study might be criticized in several respects. However, its insistence on "multiplicity" doubtless will serve to emphasize the overlap which does exist in the motor cortex and has been described, but perhaps not sufficiently emphasized, by nearly all previous students of the problem.

The authors also stress, with Walshe (65), Hughlings Jackson's dictum that movements not muscles are represented in the cortex. Whatever the meaning of this saying may be to various writers, it is nevertheless possible to analyze the basic plan of organization of the motor area in terms of the reacting muscles.

"Thus, although there is overlapping of cortical fields, for various muscles and muscle groups, comparable to the overlapping of cortical fields for peripheral cutaneous areas, individual muscles are represented maximally in specific parts of the precentral gyrus, just as areas of skin are represented maximally at particular points on the postcentral gyrus. This does not mean that a particular muscle is the only muscle represented at a specific cortical point, but that it is the one predominantly represented there" [(66), pp. 479-81].

An analysis confirming this view has been made with the aid of myographic recording by Chang *et al.* (67, 123) who found restricted regions or bands from which single muscles or a fraction of a muscle group can be activated most readily. The relationship is manifested also by threshold, tension developed, and latency and speaks for a concentration of representation of single muscles. The areas for different muscles may overlap considerably.

Thus it seems that what is needed is not to shift the emphasis from punctate to multiplex, but to recognize the fact that there are in the motor cortex, as in the postcentral and other areas, two aspects to the problem of cortical localization [compare Hines' (68) holokinesis and idiokinesis]. Furthermore, it seems unnecessary to insist that movements, not muscles, are represented, or vice versa, since movements are compounded of muscular actions in various combinations and the relation of individual muscles to the cortex can be demonstrated. Physiological factors such as facilitation, inhibition, etc., do not invalidate this view, since the whole nervous system acting dynamically makes use of stable anatomical relationships.

Bosma & Gellhorn (69, 70) studied electromyographically the effects of motor cortex stimulation on co-ordination of antagonistic flexor and extensor muscles of cats and monkeys and obtained the

following results. When tonic activity existed before stimulation, very weak cortical excitation caused inhibition of this activity in flexor or extensor or both. Poststimulatory rebound was often noted. Slightly stronger stimulation, sufficient to produce flexion or extension resulted in reciprocal innervation of agonist and antagonist but with somewhat stronger stimulation coinnervation and cocontraction occurred during flexion or extension. It was also found that a period of inhibition of tonic activity often ensued on cessation of stimulation.

Facilitation of cortically induced movements by stimulation of hypothalamic and other brain-stem centers has been studied. According to Murphy & Gellhorn hypothalamic stimulation, electrical (62), or chemical (61), produces a facilitating effect on the motor cortex itself, but Rhines & Magoun (63) obtained facilitation after complete removal of the cortex and conclude that the facilitating action occurs within the spinal cord. They found that "an uninterrupted continuity of facilitatory sites can be traced from the diencephalon backward into the lower brain stem," sites which in themselves will initiate motor activity upon stronger stimulation.

*Motor cortex: injuries.*—The disturbances of motor function without loss of consciousness observed by Russell (25) in injuries of the rolandic cortex produced by high velocity missiles are of physiological interest. In addition to paralysis and sensory loss (see p. 529) there was early loss of muscle tone and absence of tendon reflexes. Neural shock was more marked and of much longer duration in the upper than in the lower extremity, and this suggested a gross difference in degree of cortical control. It seems possible, however, to account for the difference by the more protected position of the leg area. At any rate, his case 9360, in which the leg area was undercut by a small fragment of shell, seems to indicate that effects on the leg may be as great as in the arm. While wounds of the motor cortex generally caused transient sensory loss as well as paralysis, injuries slightly anterior to the precentral gyrus occasionally resulted in severe transient paralysis without any evidence of sensory disorder.

The recovery of motor function in monkey after two-stage extirpations of areas 4 has been studied (71).

"If, after recovery from unilateral destruction of area 4, a similar operation is carried out on the intact hemisphere within one or two months after the first operation, the animal again displays a contralateral hemiparesis in all respects like

the first. If, however, an interval of three to four months is allowed to elapse between the two operations, the monkey fails to show any but the most meagre signs of pyramidal dysfunction."

To explain the less marked effect of the second operation after the longer intervals the authors suppose "there has come into existence a mechanism ready to assume the function previously exercised by area 4." From a further experiment they conclude that the postcentral gyri are necessary, since compensation does not occur in the absence of postcentral areas 3, 1, and 2. But this deduction scarcely seems warranted since the motor areas in this experiment were ablated within two months of each other, and according to the quotation cited compensation does not occur within that time.

That the time factor is important in so-called recovery of function following removal of cortical motor areas has been noted by Hines (72), and attention has been called (73) to the much better motor performance possible after bilateral 4 and 6 lesions when the cortex is removed in stages separated by considerable intervals of time. It seems likely that both cortical and subcortical centers are involved in the slow recovery process but as yet there is no good evidence concerning the contributions of each.

Watson & Kennard (74) found that recovery from unilateral 4 and 6 ablations in monkeys was retarded by phenobarbital and that dilantin when administered with doryl prevented the enhanced recovery which is caused by doryl alone (75).

A study of considerable interest is that of Sperry (76) who undertook to test the influence of horizontal intracortical organization on the control of limb coordination by making intersecting transverse and longitudinal subpial incisions at intervals of 2.4 mm. through the motor cortex. As a control the area incised was completely removed in other animals. The controls showed a severe paralysis of the contralateral arm which persisted with slow improvement for a month or longer. In contrast the partitioning incisions caused practically no disruption of arm coordination. Slight disturbances of fine movements had completely disappeared by the seventh day.

*Pyramidal and extrapyramidal pathways.*—Further studies on the pyramidal tract have been made (77 to 80), and the work which has altered conceptions of the relation of this pathway to paralysis and spasticity has been reviewed (81).

Several important papers on the pathways and centers involved

in the experimental production of spasticity have appeared. Electrical stimulation of the bulbar reticular formation (82, 83) causes general inhibition of all skeletal muscular activity. Cortical area 4S, which is known to inhibit muscular movement on excitation and to produce spasticity and hyperreflexia on removal, discharges directly into the bulbar reticular formation (84, 85) as well as into the caudate nucleus (85). Finally, evidence has been presented (86), which indicates that interruption of reticulospinal fibers in the cord produces spasticity unassociated with paralysis. Other studies report that damage to the bulbar reticular formation may be responsible for spasticity in poliomyelitis (87) and for hyperreflexia in concussion (88, 89). Magoun (90) has discussed the brain stem inhibitory mechanism in relation to the pathological physiology of Parkinson's disease and spastic hemiplegia and attention was called (89) to the fact that cerebellar inhibition operates through a bulbar relay.

It is convenient to note here reports on the use of curare (91) and neostigmine (92) in the treatment of spastic paralysis, and to cite a study (93) of muscle action potentials in human cases of spasticity and rigidity.

#### CEREBELLUM

A valuable short review of the comparative neurology of the cerebellum has been written by Larsell (94). In addition to a survey of morphological papers he includes an appraisal of the recent oscillographic investigations which have demonstrated that the cerebellum receives afferent impulses from tactile (95, 96), visual (95), and auditory (95) receptors, as well as from vestibular (97) and proprioceptive (98) endorgans. Fulton (99) also has discussed the functions of the cerebellum in relation to these newer studies. Snider & Stowell (100) have made a further report on the tactile areas of the cerebellum, comparing the cat and monkey. The representation of the ipsilateral cutaneous surface in anterior lobe and lobulus simplex is similar in the two animals, with hindlimb localized in the centralis, forelimb in the culmen, and head and neck in the lobulus simplex. Moreover, in both species the cutaneous surface is bilaterally represented also in the paramedian lobules. In cat localized potentials were recorded from paramedian lobules only when the limbs were stimulated, but in monkey it was possible to demonstrate in great detail representation of the entire tac-

tile surface of the body. The localization was found to be as follows: face in superior, arm in middle, and leg in inferior paramedian folia. It is unlikely that cat and monkey differ fundamentally, and one may expect further study to demonstrate that tactile impulses from the face also reach the upper paramedian folia of the cat. As noted below, electrical stimulation of these folia causes movements of the face (101).

The dual projection of the cutaneous tactile system to the anterior lobe-lobulus simplex and to the paramedian lobules appears to be a cerebellar arrangement corresponding to the dual sensory systems of the cerebral cortex (7). Adrian (96) has found that the face, arm, and leg subdivisions of the rolandic cortex, when strychninized, activate respectively the face, arm, and leg areas of the contralateral lobulus simplex and anterior lobe in monkey, and in the cat it is possible to show (7) that the face, arm, and leg subdivisions of the second somatic area are interrelated bilaterally with the corresponding subdivisions of the paramedian lobules. It seems likely that further study will demonstrate that subdivisions of visual and auditory areas of the cerebellum can be related to the dual auditory and visual systems of the cerebral cortex.

These specific interconnections of cerebrum and cerebellum contrast with certain earlier reports (102, 103), but it is likely that the two sets of results, obtained by different procedures, merely reflect two aspects of cerebellar organization similar to those discussed elsewhere in this review for sensory and motor cortex organization. The same statement applies to the results described in the following paragraphs which differ from earlier findings on the effects of cerebellar stimulation.

The effector systems of the cerebellum also have been reinvestigated during the past two years (101, 104) with results that correlate with the plan of somatotopic organization already revealed by afferent studies. The cerebellar cortex was stimulated through bipolar electrodes with faradic or 60-cycle alternating current of threshold strength in decerebrated cats, dogs, and monkeys after the animals had recovered from ether or pentobarbital anesthesia. Under these conditions and with care to maintain an active cerebellar circulation, a reproducible detailed pattern of localization was revealed in the three species studied. There is a rostrally located effector system occupying lobulus simplex and anterior lobe. Movements of head, including facial and masticatory muscula-

tures, are activated from the lobulus simplex, forelimbs from the culmen, hindlimbs from the centralis, and tail from the lingula. In the limb areas stimulation of the medial part of the anterior lobe evoked active ipsilateral flexion together with inhibition of antagonistic extensors. From the lateral part of the culmen and centralis the principal effect during stimulation was active ipsilateral extension. These results often were followed by the opposite actions as rebounds; also reciprocal movements in the opposite limbs frequently occurred as accompaniment of the direct and rebound effects. Caudally there is a second somatic effector system in the paramedian lobules and vermis. Movements of facial musculature were evoked from upper folia, of forelimbs from middle folia, and of hindlimbs and tail from lower folia of the paramedian lobules. The effects during stimulation were active flexions or extensions of the limbs, usually ipsilateral but sometimes bilateral. Rebound contractions and inhibitions were less regularly seen than in the rostral system. Similar movements of limbs and tail, generally bilateral, were evoked by stimulating the pyramis. From the folium and tuber vermis movements of head and eyes to the side of stimulation were regularly elicited, usually without other effects. Parts of the cerebellum not mentioned were not examined fully.

Several features of the above investigations may be emphasized: (a) the clear-cut pattern of somatotopic localization in the cerebellum; (b) the dual nature of the cerebellar afferent and efferent systems; and (c) the essential similarity of the afferent and the efferent cerebellar localization patterns. Although the inhibitory effects of cerebellar stimulation are well known it is not so generally recalled that phasic movements also occur (105).

Moruzzi (106, 107, 108) also has recently described phasic activity ("clonic movements and sometimes generalized epilepsy") produced by stimulation of the cerebellar cortex in cats under light chloralose anesthesia. The effects were abolished by pre-collicular decerebration and by bilateral destruction of the cerebral motor areas. On the other hand, inhibition of postural reflexes by cerebellar stimulation (anterior lobe and pyramis) was not affected by these operations. He concluded that phasic effects of cerebellar stimulation were related to motor cortex activity and that the general failure of previous workers to produce phasic effects by cerebellar stimulation was due to depression of the motor cortex by the anesthetic or to removal of the cerebral



cortex in precollicular preparations. However, as we have just seen, phasic movements both of flexion and of extension may be evoked by stimulating the cerebellar cortex in decerebrate animals in which the depressant action of the anesthetic agent has disappeared. Therefore, Moruzzi's conclusion that the excitatory effects of stimulating the cerebellum are due to the arrival at the motor cortex of facilitating volleys from the cerebellum needs modification. It seems reasonable to suggest that in the presence of even a mildly depressant anesthetic agent a facilitating action of cerebellar stimulation via pathways through the cerebral cortex may be required but that after elimination of even more depressant drugs phasic movements can be elicited in the absence of the cerebral circuit.

Carrea (109) has studied the effects of localized bilateral cerebellar ablations in the primate. Disturbance of equilibrium was found to follow removal of flocculus, or nodulus, uvula, and lingula. Symptoms were more marked if the paraflocculus was also removed but removal of this alone was without effect. Ablation of the rest of the vermis produced transient trunk ataxia and fine, rapid static head tremor. Removal of pyramis, paramedian, and ansiform lobules produced transient weakness, decreased resistance to passive movement, and reduced motor performance of hindlimbs. Ablation of the culmen and lobulus simplex yielded dysmetria and intention tremor in the upper limb and transient reduction in motor performance of the leg. As might be expected from the placement of the lesions these results cannot be related too clearly to the pattern of localization described above, with the possible exceptions of the head tremor following lesion of the upper vermis and the forelimb symptoms after removal of the culmen and lobulus simplex. A study in which lesions are placed according to the plan of somatotopic localization should now be carried out.

An anatomical study of importance to students of cerebellar physiology is that of Brodal & Jansen (110) on the pontocerebellar connections in the rabbit and cat. With the reservation that their lesions in the cerebellar cortex are rather less precisely placed with respect to various cerebellar subdivisions than desirable and so render the material less valuable than it might have been, one may note their conclusion that all parts of the cerebellar cortex, with the probable exception of the flocculonodular lobes, receive pontine fibers. A good many of these fibers go to the vermis but far less

abundantly than to the ansoparamedian lobule. A considerable mass of pontocerebellar fibers goes to the paraflocculus and originates in a comparatively circumscribed area of the opposite lateral gray of the middle part of the pons and from the lateral part of the paramedian gray, rostrally. The pontocerebellar connections are partly homolateral and partly contralateral. The homolateral connections are proportionately more prominent in the vermis. The major cerebellar subdivisions receive the bulk of their fibers from distinct but rather diffusely delimited areas. This is in contrast with the sharper localization revealed by study of the olive-cerebellar connections in the same material (111).

With respect to Edinger's concept of neo- and paleocerebellum Brodal & Jansen suggest that it may be most practical to reserve the term neocerebellum for those parts which are characterized not only positively by receiving pontine fibers predominantly, but also negatively by the absence of spinocerebellar and vestibular fibers as well. The neocerebellum, they suggest, thus would comprise Ingar's lobus medius, lateral parts of the anterior lobe and paraflocculus. However, it is unlikely that this definition of the neocerebellum will stand, for already oscillographic studies (95) show that more than corticopontine fibers project to lobus medius. There are connections from the trigeminus to the lobulus simplex which must be of cognate significance with the spinocerebellar connections to the anterior lobe and there are auditory and visual connections which do not involve the corticopontine circuit. Snider & Stowell's findings also indicate that spinocerebellar fibers reach the lateral parts of the anterior lobe. It seems likely then that neo- and paleocerebellum no longer define in mammals separable portions of the cerebellum unless an exception exists in the flocculonodular lobe.

It may be noted both from the oscillographic and from the stimulation experiments that the lobus medius (situated between primary and prepyramidal fissures), in its vermal portions, is devoted to functions of the head and accordingly does not receive spinocerebellar fibers. If this relation to the head should apply also to the lateral extensions of this lobe (Crus I and II), as seems to us likely, then a new conception of the functions of Crus I and II must be entertained. The importance of this would be great for the human cerebellum, where the middle lobe accounts for a large part of the total cerebellum. The experimental work indicates that

this lobe has afferent and efferent connections with the head, including eyes and ears. It is no doubt significant that this lobe is large in man where functions of the head (vision, hearing, and speech) are of paramount importance. This view also accounts for the lack of spinocerebellar connections to the middle lobe, since comparable connections come from cranial levels. The bearing of these considerations on views of cerebellar lobulation should be contemplated.

One is forced also by recent studies to reconsider the homologies of the caudal part of the human cerebellum. Thus Scholten (112) from a study of fissural development concludes that the paraflocculus of subprimates is homologous with the human lobulus biventer and the tonsil.

Our own unpublished views concerning these homologies, based on physiological data (95, 96, 100, 101, 104) and on study (with W. F. Kremer) of the cerebella of man, monkey, cat, dog, sheep, pig, rabbit, and opossum, are as follows:

The tonsil in man is the homologue of the subhuman paraflocculus. The paramedian lobule, usually homologized with the tonsil, corresponds to the lobulus biventer and the lower part of the inferior semilunar lobule of man. By analogy with cat and monkey the somatotopic localization within these paramedian homologues then would be: leg in the lower division and arm in the upper division of lobulus biventer; face in the lower part of the inferior semilunar lobule. The latter is a part of lobus medius. The rest of lobus medius is composed of the rostral part of the inferior semilunar lobule (Crus II), the superior semilunar lobule (Crus I), and the posterior part (lobulus simplex) of the quadrangular lobule.

An interesting deduction to be drawn from these identifications (in contrast with the current view, for which man appears to be the main supporting instance) is that the paramedian lobule has progressively enlarged in the phylogenetic series. This may provide a clue of physiological significance, if the preliminary studies on the interrelations of paramedian lobule and somatic area II prove correct (7).

Two papers which may be considered in relation to the cerebellum have to do with the inferior olive (113, 114) of the cat. Removal of this structure (113) produced abnormal laryngeal movement, intention tremor of axial musculature, hypermetria, and extensor hypertonus of the extremities, effects which, as the authors remark, resemble the symptoms of cerebellar ablation in carnivores and are in keeping with the fact that the olivary outflow

passes almost entirely to the cerebellum. The authors suggest that "in the absence of olivary contributions, the cerebellum would seem incapable of exerting its normal role in the integration of posture and motion."

The electrical activity of the inferior olive following spinal afferent stimulation was recorded with the aid of needle micro-electrodes (114). A variety of transection and stimulation experiments indicated that the earliest and largest activity in the olive was initiated through impulses ascending in dorsal spinocerebellar fibers or in a parallel contiguous, large fibered system.

#### MIDBRAIN, MEDULLA AND SPINAL CORD

*Midbrain.*—An interesting study on the control of eye movements by the anterior corpus quadrigeminum was made by Apter (115) on the cat. By combining local strychninization of the anterior corpus with photic stimulation of either eye she found that reflex movements of the eyes occurred and that the resulting direction of gaze was dependent on the site of strychninization. Thus a map was obtained which agreed well with that determined by the evoked potential method for the projection of the visual field on the anterior corpus quadrigeminum (116).

Kelly, Beaton & Magoun (117) abolished or greatly reduced facio-vocal behavior in cats by destroying the periaqueductal grey matter and adjacent tegmentum beneath the anterior corpus quadrigeminum—structures from which facio-vocal responses had been elicited in an earlier study by electrical stimulation.

A study of interest in relation to the problem of recovery of function in the nervous system is that of Maling & Acheson (118, 119) on the effects of *d*-amphetamine on acute low-decerebrate and spinal cats. The resulting functional status was comparable in many respects to that of long-surviving chronic preparations (120).

A monograph on decerebration in man (121) has been published but was not available for comment.

*Medulla and spinal cord.*—Bodian (122) investigated the spinal projections of brain-stem nuclei in monkey by the chromatolytic method after hemisection or transection of the cord at various levels. Quantitative data were secured on the pars magnocellularis of the nucleus ruber, Deiters' nucleus, midbrain tegmentum, and hindbrain reticular formation. Wagley (86) also studied brain-stem nuclei after partial sections of the cord in his analysis of spinal tracts concerned in spasticity and paralysis.

An important investigation on segmentation, lamination, and topographical projection in the nervous system with reference to the tail of the spider monkey was made by Chang (123). Several features of this study are of especial interest. Fibers in the ventral spinocerebellar tract were traced from caudal cord segments to the lingula, lobulus centralis, and lips of fissura secunda of the cerebellum—findings which fit well with the plan of afferent and efferent localization in the cerebellum. Part of the spinothalamic tract fibers cross through the posterior commissure to the nucleus ventralis posterolateralis of the thalamus providing, as Chang suggests, a potential pathway for ipsilateral sensory representation in thalamus and cortex. A hitherto undescribed spinal component of Gudden's supraoptic commissure also was reported (123, 124).

From a study of Ranson-Cajal series of spinal cords of sheep and rats Sprague (125) presented evidence against the generally accepted view that the motor cells of the spinal cord may be separated into two groups—a medial one for axial and a lateral one for limb muscles. He concluded that unless considerable shifting of neurons occurs in later development all motoneuron groups contribute innervation to both. Bernhard & Rexed (126) found that the internuncial neurons which most easily discharge into the peroneal nerve of the cat are located in the lateral part of the intermediate zone and that stimulation of the medial part activates peroneal motoneurons only at greater stimulus strength. They suggested that the medial part may discharge optimally to trunk musculature. It would be worthwhile to check by electrical methods the amount of overlap in the anterior horns of the motoneuron pools supplying axial and appendicular musculatures.

Campbell (127) reported that chromatolysis of anterior horn cells is characterized by a decrease in irritability and not by a loss of "specific transmissive functions."

Cannon *et al.* (128) compared the results of transecting the spinal cord of normal cats with the effects obtained when the transection followed by eight to twenty-five days a previous hemisection. They concluded that hemisection hypersensitizes some neurons in aboral segments to both the stimulating and the inhibiting effects of afferent nerve impulses. The hypersensitivity was bilateral but greater on the side of hemisection and was attributed to partial denervation. Three other studies of reflex activity in spinal or decerebrate cats may be noted (129, 130, 131).

Attention is called to excellent critical reviews by Straus (132)

on the concept of nerve-muscle specificity and by Sperry (133) on the problem of central nervous reorganization after nerve regeneration and muscle transplantation.

#### MISCELLANEOUS

In addition to the work already considered the following contributions may be listed. Howell's *Textbook of Physiology* (99) has been revised with the creation of an essentially new text, of which a large part is devoted to the nervous system. Herz & Putnam (134) have offered an atlas and syllabus of teaching films in clinical neurology. Contributions appeared on the functions of man's frontal lobes (135, 136). The pattern of the primate cortex was studied in *Galago* (137). Krieg described the craniocerebral topography of the monkey (138) and offered a stereotaxic instrument designed for the rat together with a brief atlas of cross sections of the rat's brain (139). He also illustrated in a most useful manner the topography of the spinal cord and vertebral column of the cat (140). Mettler (141) described the effects of removing the neopallium and striatum and Dandy (142) proposed the striatum as the center of consciousness. Hypothalamic attacks with a lesion of the thalamus (143, 144), and clinicopathological studies (145 to 149) on disturbances of sleep were reported. Finally a valuable paper on housing, care, and surgical handling of laboratory primates (150) appeared.

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DEPARTMENT OF PHYSIOLOGY  
SCHOOL OF MEDICINE  
JOHNS HOPKINS UNIVERSITY  
BALTIMORE, MARYLAND

## SPECIAL SENSES, CUTANEOUS SENSATION<sup>1</sup>

BY F. K. SANDERS

*Department of Zoology and Comparative Anatomy, University Museum,  
Oxford, England*

Papers published recently on cutaneous sensation fall into two main groups: (a) fundamental work on the anatomy and physiology of normal cutaneous sensibility; and (b) investigations into the pathological manifestations of sensibility accompanying injury and other clinical conditions, which throw light on various aspects of sensory physiology. The literature covered is that of the war years, duplication with earlier articles in this series (18, 49, 50, 91) being avoided insofar as is possible; key references to earlier work have also been included. For further references the reader is referred to reviews recently published by Bishop (12), Dallenbach (17), Walshe (75) and Weddell (82). Owing to the continued difficulty of obtaining many journals, it cannot be claimed that a complete survey has been made.

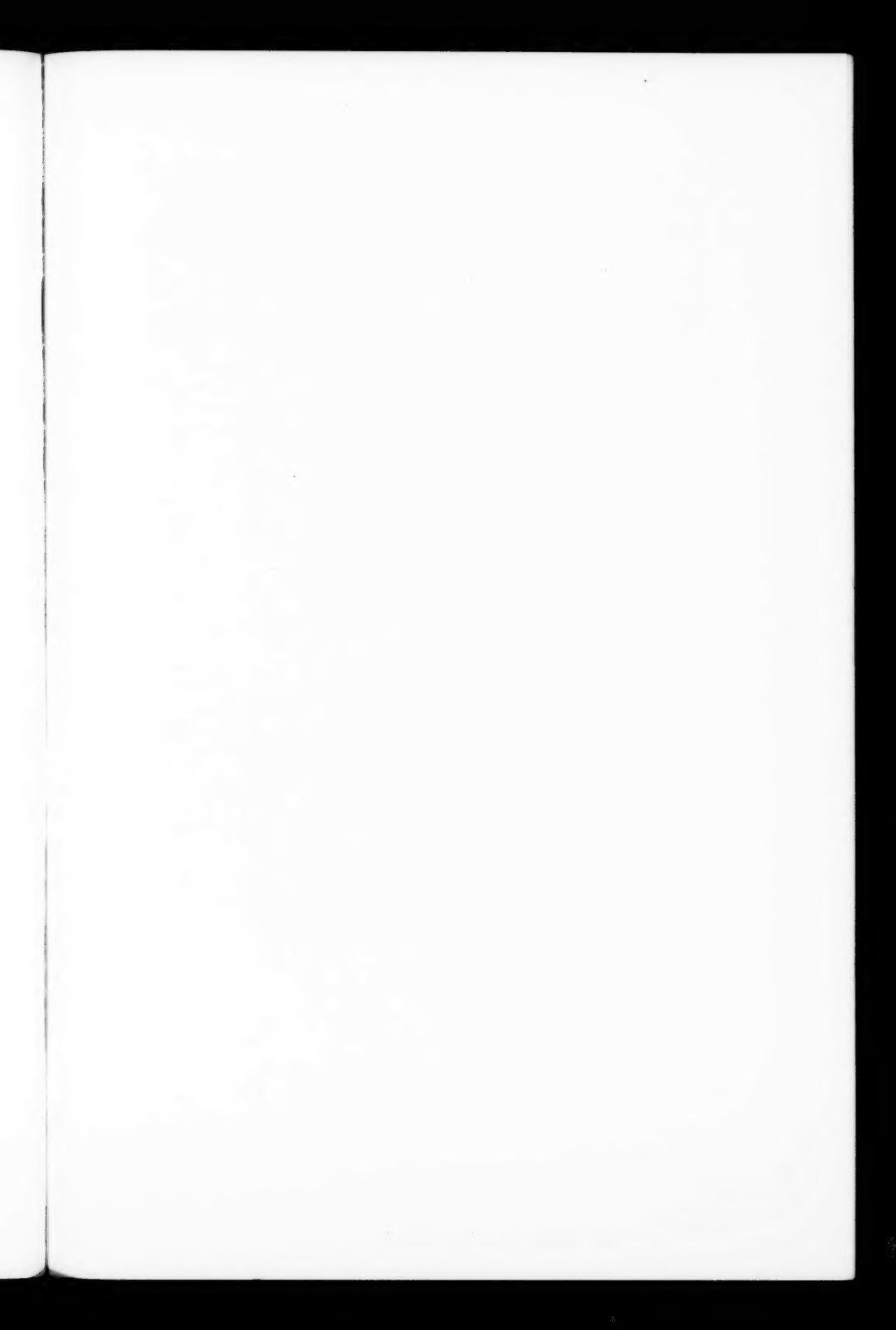
*Senses mediated by the skin.*—A large number of distinct and readily recognizable sensations can be aroused from the skin of man. In number these exceed the number of discrete types of cutaneous nerve endings identifiable histologically. The skin is therefore regarded by most workers at the present time as mediating only four main modalities of sensation, namely, touch (including both light touch and pressure), cold, warmth, and pain (17, 82, 86). Tickle and itching have been ascribed to a characteristic pattern of response in the pain modality (9, 10, 63, 64) while more complex sensations, such as roughness, wetness, and vibration, are considered as resulting from a complex of stimulation in two or more modalities (82). There being centrally the ability to discriminate subjectively these four modalities of sensations, four corresponding classes of endings in the skin are inferred, responding typically, but not exclusively, to different forms of stimulation. Bishop (12), in an admirable review of the whole field, points out that the modality concept, being differentiated on a dual subjective and objective basis, can lead to some confusion if strictly applied. For not only do more than four types of endings exist in the skin, but various types of stimulation of a single

<sup>1</sup> This review covers the period up to August, 1946.

sensory "spot" in the skin can arouse different sensations, for instance the paradox of nonpainful pain on weak stimulation of pain endings (10). However, the modality concept has a great deal of usefulness as a loose-fitting generalisation, and probably has some objective basis, since ascending pathways within the spinal cord segregate roughly according to modality (74).

A second characteristic of skin sensibility is that it is punctate in distribution. For all modalities, except possibly warmth, each skin area consists of a mosaic of highly sensitive points, each surrounded by a relatively insensitive zone (9, 10). The sensitive points for the different modalities do not necessarily coincide.

*Anatomical basis of cutaneous sensibility.*—The anatomical basis of skin sensation consists of specific punctate endings lying at different depths below the epidermis and grouped into areas (or interlocked in the case of pain endings). To permit each spot to be innervated by a number of endings derived from separate nerve fibres, there is a complex scattering of nerve fibres beneath the skin. This picture of skin innervation is chiefly due to recent work by Woollard, Weddell, and their collaborators (78 to 84, 89). By the examination and comparison of the neurohistology of skin taken from animals, normal humans, and patients with dissociated sensibility resulting from nerve injuries, it was possible to specify the nerve endings giving rise to sensations of different modalities. In this way cold sensibility is referred to Krause's end-bulbs, while the Ruffini endings may be the receptors for warmth. Pacinian corpuscles and the Golgi-Mazzoni endings are pressure receptors. The remaining modality, cutaneous pain, is served by "free" nerve endings arising from both medullated and non-medullated nerve fibres arranged in a plexiform interlocking manner. By the study of large whole mounts of skin stained intravitaly with methylene blue it has been possible to study the disposition within the skin of the nerve fibres supplying the above end-organs. Weddell (78) showed that there is a constant pattern of skin innervation in *Acanthias* (an elasmobranch fish), rabbit, monkey and man. A constant feature of this pattern is a cutaneous nerve plexus, in which fibres interdigitate but do not fuse with one another, disposed in two main layers through the thickness of the skin. As one ascends the series, however, there is a great increase in complexity as regards the variety of end-organs encountered. The features of the plexus have been worked out in the greatest



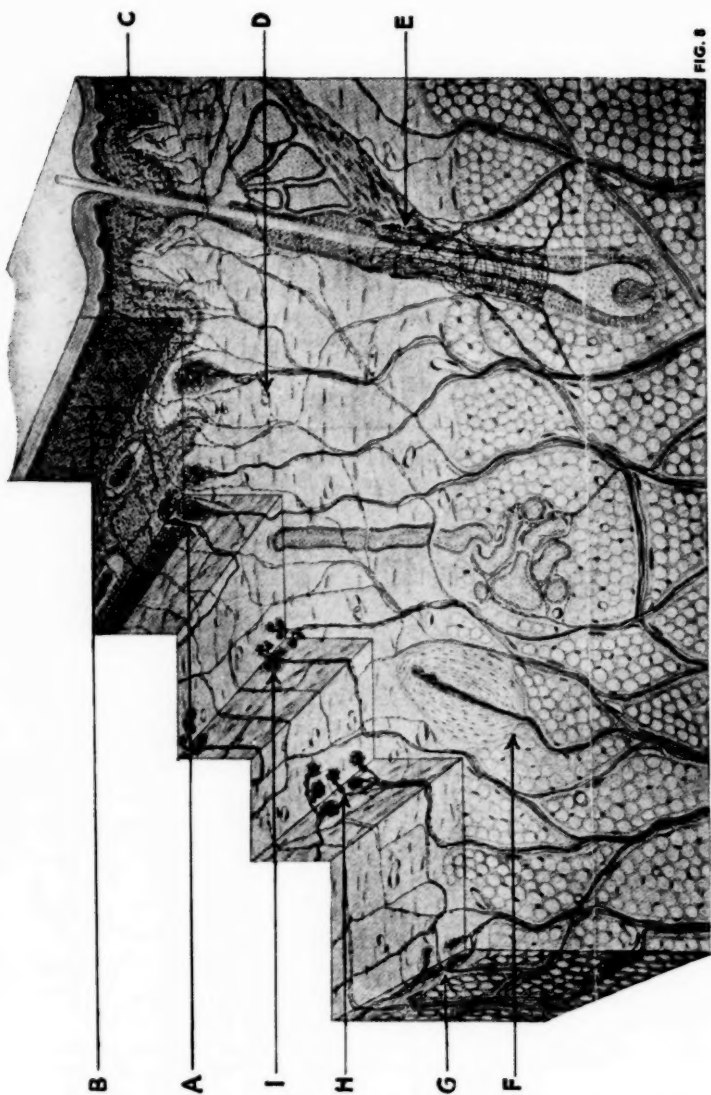


FIG. 8

Diagram of Cutaneous Innervation according to Weddell (82). A, groups of Meissner's corpuscles subserving the sensation of touch; B, beaded nerve fibres subserving pain; C, Merkel's discs subserving touch; D, beaded nerve fibres derived from nerve nets subserving pain and associated with blood-vessels; E, nerve terminals around the sheath of a hair subserving touch; F, a Pacinian corpuscle subserving pressure; G, a group of Ruffini endings subserving warmth; H and I, groups of Krause's end-bulbs subserving cold (these lie at somewhat variable depths below the skin surface). The organised endings are accompanied in every instance by fine beaded nerve fibres.



detail in the skin of the dorsum of the rabbit's ear (78, 81). Here the branches of the nerve trunks approaching the skin do not run in parallel formation, but turn and inosculate with one another in a manner resembling the reticulate venation found in the leaves of some plants. A similar arrangement of nerve trunks seems to be a general feature of mammalian skin (79, 80, 81). Thus, in general, each unit area of skin is evenly innervated by fibres approaching it from all directions. An obvious corollary of this state of affairs is that small wounds of the skin cannot produce extensive areas of anaesthesia.

After leaving the cutaneous nerve trunks, nerve fibres enter the cutaneous plexus where they may undergo extensive branching, there being little or no branching within the cutaneous trunks (78). This plexus consists of the interdigitation of two layers of dichotomised nerve fibres, arranged in meshwork patterns enclosing polygonal areas. In the rabbit's ear the centre of each polygon is occupied by a group of hair follicles. Fibres supplying the various types of ending can be traced back into the cutaneous nerve plexus. This picture of skin innervation is summarised in the figure on the adjacent page.

Branches of a single nerve fibre, traced through all their ramifications in the cutaneous nerve plexus, were found to bear endings of one type only (82). Following partial section of the dorsal ear nerve of the rabbit, single fibres in the early stages of degeneration stand out clearly from their background, and can be traced for long distances. In this way it was found that the terminal branches of single fibres innervating hair follicles were distributed over wide circular areas approximately 1 cm. in diameter. Each fiber innervated as many as three hundred follicle groups. Each follicle group was supplied by branches from at least two main fibres. The same also applied to individual hairs, each of which may bear terminal ramifications from as many as fifteen separate nerve fibres (78, 82). Similar conditions were found to obtain in the case of Meissner's corpuscles and Krause's end-bulbs. Within groups of these endings different corpuscles or sets of corpuscles were innervated by fibres approaching the group from different directions (79).

The fine myelinated and nonmyelinated fibres subserving pain give rise, on emerging from the cutaneous nerve plexus, to fine terminal ramifications, or "nerve-nets" (89). These in their turn

give rise to "free" endings, often beaded in appearance in methylene blue preparations. The nerve nets from a single fibre are distributed over an approximately circular area, and the fibres from which they arise remain single and unbranched as far as they can be traced through the cutaneous nerve plexus. Nerve-nets and free endings occur in two main sites: (a) in the deeper layers of the epidermis; and (b) in the sub-epidermal tissues, associated with blood vessels. The nerve-nets with intraepithelial endings may connect exclusively with myelinated nerve fibres (42). The nerve-net derived from any one fibre is interlocked with those arising from neighbouring fibres, but there is no demonstrable fusion between the two (78, 89). Finally, all the more complex endings, such as Pacinian corpuscles, Krause's end-bulbs, Meissner's corpuscles, etc. have accessory endings of the same morphological type as the nerve nets, and supplied by similar fibres (89). Similar accessory fibres are found in the more complex sensory end-organs of deeper tissues, such as muscle spindles and Golgi tendon organs (83), and probably mediate pain from these sites (89).

The areas of skin supplied by adjacent cutaneous nerves overlap to a varying extent. Thus when a nerve supplying a particular area of skin is severed, an anaesthetic zone is produced, surrounded by a further zone in which there is a diminished number of sensory "spots," and from which the quality of sensation aroused is abnormal (see below). This is the so-called intermediate zone, which is supplied with nerve fibres partly by the severed nerve and partly by adjacent nerve trunks. Some time after nerve severance there is often a shrinkage of the anaesthetic zone in the absence of regeneration of the severed nerve. It has recently been shown that this results from the outgrowth of nerve fibres from the intermediate zone through the cutaneous nerve plexus into the denervated area (84). Whether this outgrowth takes place by new branches which sprout from otherwise normal fibres in the intermediate zone, or by regenerative growth of damaged fibres in nerves adjacent to the severed nerve, is uncertain (84). Normal skin, especially in regions constantly exposed to minor trauma, such as the elbow, frequently contains a proportion of nerve fibres undergoing degeneration and regeneration (84), and it may be these that provide a source of new fibres which can regenerate into the anaesthetic zone.

In the above account of skin innervation no mention has been made of sudomotor and pilomotor innervation, or of the fibres

which innervate cutaneous blood vessels. The low value normally assumed by electrical skin resistance depends on the presence of an intact sudomotor innervation, and this property has been made use of in devising an objective method for (a) mapping areas of sensory loss after nerve lesions, and (b) distinguishing between cutaneous insensitivity due to nerve lesion and that due to hysteria (37, 38, 61). Apart from these and the system already discussed, there is as yet no anatomical evidence for the special cutaneous nerve fibre systems (epicritic and protopathic) postulated by Head (34, 75) and by Lewis (43, 89). These phenomena are further discussed in a subsequent section.

*Physiological characteristics of cutaneous afferents.*—The compound action potential of cutaneous nerves contains beta, gamma, delta, epsilon, and C waves, each referable to different maxima within the fibre-diameter spectrum (12, 25). The absence of an alpha wave can be correlated with the lack of the separate group of large fibres, present in the fibre-size spectrum of muscular, but not of cutaneous branches (66). Although the different diameter groups are distinct as regards their maxima, they shade into one another at their margins. Fibres of different sizes contributing to distinct parts of the compound action potential, differ also as regards accommodation, threshold, and susceptibility to asphyxia and narcotics (12, 25, 40), and procedures making use of the last three properties have been used to correlate different fibre groups with different modalities of sensation (8, 10, 26, 43). So differentiated, fibres subserving different modalities have in no case been found to be restricted to a narrow range of fibre-sizes (12, 25). Furthermore, in no respect have all the fibres of any one skin nerve been specified by such means. However, it has been possible to refer different modalities of sensation broadly to different parts of the action potential. Touch, in general, seems to be a property of the largest myelinated fibres, although it may also have a component in the delta group (12, 25). It is interesting to note in this respect that the existence of two distinct kinds of touch—one from hair follicles and the other from the areas of skin between them (9)—may be correlated both with two distinct types of ending (see above) and with a differentiation with respect to fibre size.

Pain is mediated by two distinct sets of fibres, one situated at the head of the delta group, and the other nonmyelinated and in the C range. Here again there is a correlation with the anatomical

data (see above). The difficulty at present is to correlate this very distinct specificity as regards fibre-type with the two pain system reported as a result of experiments on human subjects. Lewis and his collaborators have now established the reality of the double pain response to pinprick. The two phases of the response—the initial, bright prick, and the later, longer-lasting, aching pain—are separable by differential asphyxiation and cocainisation, and these same authors have shown that the differences in reaction time to the two pains are consistent with the known differences in conduction rate between delta and C fibres (43). This conclusion has been amply confirmed by Gordon & Whitteridge (26) recording the reaction times to the two pains by the disturbances they induce in the alpha-rhythm of the electroencephalogram. Electrical stimulation of pain spots by a high-voltage spark, however, induces only one sensation, identified by Bishop (9, 12) with delta pain. Moreover pain of the same aching quality as "second" pain can be induced by repetitive stimulation of such a spot, under conditions where single shocks of the same intensity give only bright prick (9). Wolff and his co-workers (8, 86) also distinguish two pain sensations on stimulation of the skin with radiant heat under controlled conditions. On increasing the strength of stimulation the sensation of warmth changes suddenly into a bright pricking pain at a threshold which is remarkably constant in the individual, and has a total range of variation of only plus or minus 15 per cent about the mean threshold in a large number of normal subjects (15). On further increasing the intensity of the applied radiation the pricking pain merges into a gradually increasing intensity of burning pain. The thresholds of the two pains vary independently under different conditions (8). Pricking pain has a lower threshold than burning pain and under partial cocaine analgesia burning pain is abolished while pricking pain remains. Ischemic block of an extremity, on the other hand, initially lowers the threshold of both pains. That for burning pain is more depressed and remains so for far longer than pricking pain. Also during the period of depressed burning pain-threshold, the sensation of "burning" lasts much longer than normally after the stimulus is removed. Finally, in prolonged ischemia both thresholds are raised and complete analgesia ensues. In view of these results it is extremely tempting to conclude that Lewis' fast pain, Wolff's "pricking pain" and delta pain are equivalent, while slow, burning, and C pain are similarly identical. However, so long as our knowledge in these

matters depends ultimately on a subjective judgment of sensation quality, their identity, or moreover, the differences in sensory response to delta and C fibre stimulation cannot be said to have been unequivocally demonstrated. Indeed, as Bishop (12) has pointed out, two separate phenomena may have been confused here in the past: an apparent double pain when touch and delta fibres are simultaneously discharged, the initial touch being interpreted as pain when associated with delta responses; and a true double pain, due to combined delta and C responses. Which of these two is elicited in a particular case may depend on the intensity and kind of stimulus.

*Physiological differences between cutaneous endings.*—Types of endings can be differentiated on the basis of differences in adaptation and after-discharge, but so far there have been few attempts to discover the properties in this respect of specific anatomical types of endings. Even for the major modal classes relatively little information is available.

Tactile endings adapt with extreme rapidity and completeness, have no after-discharge, and show on-off effects reminiscent of retinal afferents (4, 12). They can follow the frequency of a repetitive stimulus to the maximum frequency of which the particular receptor is capable. Pain endings, on the other hand, adapt extremely slowly, and may show the converse, namely an augmentation of the discharge with continuation of the stimulus (4, 25). However, the epithets "bright," "stabbing," "pricking," applied to what is presumably delta pain, may imply some power of adaptation. Pacinian corpuscles adapt less rapidly than tactile receptors (5). In these data there is an obvious correlation between the activity of the different sense organs and the type of information they convey. For example, touch receptors would be unable to convey a vibratory sense if they were not "dead-beat." Similarly the purpose of pain endings would be vitiated if the ending ceased to discharge before the offending stimulus was removed.

Not enough is known of the essential character of sensory excitation to enable us to correlate the specificity of endings with their structure. It may, however, be noted that pain is the only modality served by free endings, while all the other modalities depend upon endings which are variously capsulated and enclosed (see above). This anatomical difference is presumably correlated with the ability of a great variety of stimuli to cause pain, whereas all the other types of ending are more or less specific for a particular

class of stimulus. In the case of the vibratory sense it is deformation of the skin rather than change of pressure which activates the appropriate endings (52) and the intensity ascribed to the stimulus is determined by acceleration and not by rate of displacement. As a result of this, a subject can discriminate between rectangular and sinusoidal oscillations independently of their frequency.

*Localisation: the sensory unit.*—Recent evidence makes it unlikely that precise cutaneous localisation of a stimulating point depends on simple point-to-point connections between skin surface and cortex. For it has been shown that cutaneous stimuli are, in general, localisable within an area smaller than that covered by the terminal ramifications of a single nerve fibre. Tower (73) has shown that one fibre may supply a complete quadrant of the cornea, an area about 1 cm. in diameter. Similarly, in the rabbit's ear a single fibre can also supply an area of this diameter (78), while in skin from a human forearm an isolated pain nerve-net was found whose greatest diameter 7.5 mm. (78). Tactile units with an area varying from 100 sq. mm. [in frog skin; (4)] to 5 sq. mm. [in the cat's tongue; (55)] have also been reported. In regions of precise localisation the area occupied by a single pain-net was smaller, being 1.5 mm. in diameter in the monkey's thumb pad (78). In the touch modality also there may be as many as ten groups of Meissner's corpuscles per sq. mm. in the human finger (79).

Such findings have led Tower (73) to construct a theory accounting for spatial localisation of pain in terms of the central analysis of patterns of activity in overlapping neuron units, which may be profitably applied to other modalities. One advantage of this theory is that it accounts for the diminished accuracy of spatial localisation observed at low intensities of stimulation (10). It now appears that at least three varieties of "sensory unit" can be distinguished: (a) the anatomical unit, consisting of all the terminal ramifications of a single nerve fibre; (b) the physiological unit, which is the lower limit of area the position of which on the body surface can be accurately specified; and (c) the sensory "spot" characterised by a central point of maximal sensitivity [which may be anatomically the point at which a nerve twig from the cutaneous nerve plexus turns up towards the surface of the skin (11)] surrounded by a less sensitive area.

*Central representation.*—In the case of tactile sensibility succes-



sive regions of the body have discrete cortical representation (74). A greater proportion of the tactile area of the postcentral gyrus is devoted to that part of the body which is most used by the species concerned in exploring its environment (1, 2, 3). Touch from hairs has also regional representation in the cerebellum (69). Evidence for similar cortical and cerebellar localisation of pain is lacking. That pain impulses do, however, reach the cortex is shown by the abolition of a painful phantom limb by excision of the contralateral postcentral cortex (32). Walker (74) has summarised the central pathways and areas involved in pain perception and concludes that integration can occur at three levels: (a) the cortex, where the appreciation of pain may be coloured by associated tactile and pressure impulses, and be influenced by the activity of other cortical centres; (b) the thalamus, whose function is not the appreciation of pain, but its integration with other modalities; (c) the tectum mesencephali, the primitive pain centre where appreciation of a low order—a "feeling tone" may occur. It has also been claimed that specific centres exist in the cortex for such complex sensations as itch and tickle (90).

The fact that the perception, and, possibly the quality of cutaneous sensation is as much a function of the centre as the periphery has been stressed by many observers, most recently by Bishop (12). Walker (74) describes a case of phantom limb pain relieved by bilateral frontal lobotomy, where, although the pain was still present after operation, the patient was unable to direct her attention to it, so that it ceased to dominate her sensory experience. Kunkle & Chapman (41) describe a case of almost complete insensitivity to pain which they ascribe to a central defect. Similarly it has been found that in hysterical anaesthesia the reflex rise in blood pressure which normally accompanies immersion of part of a limb in cold water is still present, which indicates that in this condition also the sensory pathways are not blocked at lower levels (71). Further evidence that attitude and suggestion play a considerable part in the perception and reaction to pain is shown by experiments in which it was possible to raise the pain threshold in suitable subjects as much as thirty per cent merely by the administration of placebos (87). Similarly prejudice, anxiety, and doubt, could prevent the pain threshold raising action of drugs such as aspirin. Elevation of the pain threshold could also be obtained merely by distracting the subject's attention. Sub-



stances such as morphine, physostigmine, and prostigmine also raise the pain threshold; prostigmine greatly potentiates and prolongs the analgesic action of morphine (24).

Central activity, even at the level of the cord, can also influence the localisation of peripherally induced pain. Patients with hemianalgesia following unilateral high thoracic section of the ventrolateral part of the cord perceived pain referred to the normal side when the analgesic side was intensely and noxiously stimulated (59). Spread of pain can thus occur as a result of the spread of excitatory processes within the cord. A similar process can occur at higher levels of integration. In lesions of the brain showing pain referred unilaterally to a cutaneous area, either an intensification or a decrease of the pain from the affected area can often be induced by stimulating the corresponding skin area of the normal side. This phenomenon occurs only rarely in spinal cord lesions, and is usually absent from cases of peripheral nerve injury (7). A similar mechanism may be responsible for the referred pain of visceral disease.

*Hyperalgesia and hyperpathia.*—The production of a zone of hyperalgesia surrounding a point of injury produced by crushing or other noxious stimulation of the skin has been ascribed by Lewis (43) to the presence in the skin of an arborising system of nerve fibres derived from the dorsal root—the “nocifensor nerves”; these fibres do not mediate any specific sensations, but produce hyperalgesia and vascular reactions by the release of appropriate substances into the skin. Lewis believed that the nerves concerned in hyperalgesic spread could not belong to the pain or tactile systems, since the latter sensations are accurately localisable, which he considered could hardly be the case if the impulses concerned originated in a system of branching axones covering a large area of skin. Tower's (73) theory, however, suggests that spatial discrimination need not be a function of the size of individual neuron units, and with this new concept the chief need to postulate a nocifensor system disappears. At the present time, therefore, there is little to contradict the hypothesis that pain nerves mediate hyperalgesic spread. A corollary of this, however, is that if the pain nerves are responsible for nocifensor reactions, the diameter of hyperalgesic zones should not exceed twice the diameter of the largest pain terminals. Pain terminals as large as those indicated by Lewis's demonstration of hyperalgesic zones up to 18 cm. long by 7 cm. wide after local electrical stimulation of points on the

human forearm remain to be demonstrated. That hyperalgesia is of peripheral origin is supported by recent data showing that hyperalgesia of local injury is associated with a lowered pain threshold in the hyperalgesic zone (68, 86). As such it should be separated from the so-called hyperalgesia associated with dorsal root, ganglion, or nerve disease, in which the threshold is unaltered or even raised (33), although the sensation perceived may be more intense than normal. In addition to reduced pain threshold locally induced hyperalgesia may be accompanied by local vascular changes (triple response) ascribed by Lewis to the release at the site of injury of a histamine-like substance (43). Histamine itself produces a local vascular flare about the site of injection when pricked into innervated skin, and has been used as a test for the presence of innervation (72). Hyperalgesia itself Lewis ascribes to the production of a second substance (P-P substance) at the nerve endings of the fibres responsible for hyperalgesic spread. He does not identify this substance with histamine, on the grounds that the latter, pricked into the skin in buffered solutions of low concentration, provokes itching but not pain (43). Itching itself, however, may result from the stimulation of pain endings below the intensity required to evoke pain (9). Moreover intravenous injection of 10 mg. per kg. of a powerful anti-histaminic, diethylaminoethyl-N-benzylaniline, increases tolerance to the burning pain produced by immersion in hot water, and also relieves itch and the pain of zoster (51).

Pain with intensified responses is also aroused typically from intermediate zones and during recovery from nerve lesions, having (a) increased threshold, (b) an explosive, unpleasant diffuse quality, with which is associated a desire to rub the affected part, and (c) peripheral reference, pinpricks in the proximal part of affected area being referred to its distal margin (34, 43). Similar reactions are present to a lesser degree for the other modalities, and are conspicuous in the case of cold (34). Such an area also shows a decreased number of sensory "spots" per unit area (82). Pain of the above qualities is that termed "protopathic" by Head (34), and which he assigned to a special set of nerve fibres. The evidence for the protopathic system has recently been reviewed by Walshe (75), who concludes that the whole concept of a protopathic system is untenable. The actual mechanism for the production of pain of this type, however, remains unknown. The reaction time is shorter than would be expected if the fibres involved conducted

only at C velocities (26). Moreover, Weddell (81) has shown that there is a stage in recovery when many of the normally multiple innervated groups of cutaneous endings are only innervated by a single fibre. This might account for the wrong reference and "diffuse" quality of the sensation experienced. Furthermore, it has been shown that regenerating nerve fibres have a lower threshold of mechanical excitability than normal fibres (11, 39), and thus the stimulation of fibres which have not yet reached their endings may contribute to the abnormal sensations experienced during reinnervation.

*Causalgia and the phantom limb syndrome.*—These two pathological aspects of cutaneous sensation are encountered more frequently in war than in peacetime, and a large number of clinical papers have recently appeared dealing with these subjects.

Causalgia, which occurs occasionally after traumatic partial division of a peripheral nerve has, according to recent descriptions (16, 36, 46, 48, 54, 62, 67, 77), the following features: (a) Severe burning pain of one or more extremities is present with a characteristic burning quality, from which the patients often seek relief by keeping the affected part perpetually cold and wet. (b) This pain is exacerbated by almost any stimulus, however slight, and also by anxiety or any mental upset. (c) Motor and sensory loss is minimal or absent, but the skin of the affected limb is tender, glossy, transparent, and shows vasodilatation and sometimes hyperhydrosis. (d) There are characteristic changes in the nails and the tips of the fingers or toes. Many examples are known of intractable pain following (sometimes slight) peripheral injury which do not show all of the above symptoms, and these have been separated, by Evans & Livingston (22, 44), as "minor" causalgia or "reflex sympathetic dystrophy." Similarly pain of hysterical origin does not generally show all the above symptoms although it is sometimes difficult to separate from causalgia.

The reviewer has been able to discover reports of 384 cases of severe cutaneous pain in war casualties (6, 13, 14, 22, 30, 31, 36, 46, 47, 53, 54, 57, 58, 65, 67, 80). Not all of these were causalgia as defined above, but the uniform results obtained from these cases, with a smaller number of well-documented cases of causalgia itself (46, 54, 58, 70), enables certain conclusions to be drawn as to the nature of these complaints.

Attempts to relieve causalgic pain by neurolysis, resection of

damaged nerves, or periarterial sympathectomy are frequently ineffective (36, 54, 62, 77, 85). Causalgia is, however, frequently relieved by interruption of the sympathetic trunk at the appropriate level. Of the above cases, 208 were so treated: 176 (84.6 per cent) had complete relief of pain; 22 (10.6 per cent) had moderate relief; in only 5 was the pain undiminished by this operation; the results of treatment in the remaining 5 cases are not stated. Similarly, sympathetic ganglion block with local anaesthetics frequently affords temporary relief from the pain of causalgia (30, 36, 46, 58, 70) and is often used as a preliminary indication of the probable results of subsequent sympathectomy (44, 46, 47, 62). In occasional cases, and more frequently in minor causalgia, this procedure may give permanent relief (25, 58, 70).

These results, together with the peripheral vascular symptoms, all points to the participation of the sympathetic nervous system in the genesis of causalgic pain. Livingston (44) suggests as a possible mechanism for causalgia a vicious circle of neuronal activity. Initial irritation of sensory neurons at the level of nerve lesions is presumed to start closed cycle activity in internuncial neurones. This in its turn initiates motor and sympathetic effects in the periphery which combine further to irritate the sensory system. While this provides a possible mechanism for a condition which until recently was almost wholly puzzling, yet it fails to account for such phenomena as the vasodilatation which is a common feature of causalgia, unless it is further assumed that the cord activity also stimulates antidromic vasodilator fibres. Doupe and his co-workers (19, 21), however, have recently claimed that vasodilatation may be mediated at least in part by fibres of sympathetic origin. Doupe, Cullen & Chance (20) themselves regard causalgia as the result of direct excitation of sensory by sympathetic fibres either at the site of lesion or peripherally, and propose a classification of causalgias based on this difference. Porter & Taylor (56) have described facilitation of the flexion reflex of the tibialis anticus following the application to the skin of the foot of a variety of stimuli, and it is suggested that a similar mechanism may account for the paroxysmal nature of causalgic pain following slight cutaneous stimulation.

The characteristics of the phantom limb syndrome have been reviewed by Riddoch (61). Recent papers have been chiefly concerned with the most effective procedures in relieving phantom

limb pain (23, 35, 45, 85). Falconer & Lindsay (23) point out that two main types of phantom limb pains can be distinguished—one in which the pain seems to arise in or around the neuromas and can be relieved by their excision or by sympathetic nerve block, and a second group in which the pain originates in the spinal cord. For the latter type such procedures as section of the spinothalamic tract, excision of the sensory cortex, and frontal lobotomy have been used (23, 32, 74). The first of these operations is preferable since it relieves pain without destroying the patient's appreciation of his stump.

In connection with phantom limbs the observations of Granit, Leksell & Skoglund (27, 28, 29) on the "artificial synapse" are of interest. These authors showed that impulses in ventral root fibres arriving at the freshly cut end of a peripheral nerve are able to initiate impulses in the dorsal root fibres. Whether the cut end of a nerve retains these properties after the formation of a neuroma is not known. Were this the case, a possible mechanism for the genesis of phantom limbs is provided, which would also account for the reappearance of phantom limb sensations after the neuromas thought to be responsible have been excised.

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DEPARTMENT OF ZOOLOGY AND COMPARATIVE ANATOMY  
UNIVERSITY MUSEUM  
OXFORD, ENGLAND



## THE EXPERIMENTAL NEUROSIS<sup>1</sup>

By H. S. LIDDELL

*Department of Psychology, Cornell University, Ithaca, New York*

It is now thirty years since Pavlov's attention was directed to the phenomena of abnormal behavior in animals and man. A dog, subjected to prolonged conditioning, was unable to distinguish between the appearance of a luminous circle as a signal for food and an almost circular ellipse as a signal for no food and in consequence suddenly exhibited extreme and enduring agitation. As Pavlov [(1), p. 292] describes it:

"The hitherto quiet dog began to squeal in its stand, kept wriggling about, tore off with its teeth the apparatus for mechanical stimulation of the skin and bit through the tubes connecting the animal's room with the observer, a behavior which never happened before. On being taken into the experimental room the dog now barked violently, which was also contrary to its usual custom; in short, it presented all the symptoms of acute neurosis. . . . After these experiments we paid considerable attention to pathological disturbances in the cortical activity and began to study them in detail."

From this laboratory incident eventuated a systematic program of research in Pavlov's laboratories devoted to the chronically disordered behavior of dogs subjected to difficult regimens of conditioning. Of particular importance was his discovery that "experimental neuroses are usually permanent, affecting an animal for months and even years" [(2), p. 180].

Since the observation in 1927 of experimental neurosis in the sheep by Liddell & Bayne (3) this field of investigation has been extensively explored in this country not only in the dog but in other mammals, such as sheep, goat, cat, pig, and recently, the chimpanzee. The following brief review is limited to a consideration of experimental neuroses as they have recently been observed and experimentally analyzed in the larger mammals.

Impressive literature devoted to the study of audiogenic seizures and other disorders of behavior in the white rat has accumulated. Finger [(4), p. 427] concludes his review of studies concerning experimental behavior disorders in this animal as follows:

"It is difficult to evaluate the ultimate significance of these investigations for

<sup>1</sup> This review covers the period from 1941 to 1946.

problems of human behavior disorders. The resemblance of the audiogenic seizure to certain forms of human epilepsy cannot be overlooked; our understanding of the human disturbance may eventually be advanced through the study of the rat's abnormal reaction. Moreover, the findings of these animal experiments may contribute appreciably to the theory and practice of shock therapy."

Recent advances in the investigation of experimental neurosis in the larger mammals mentioned above have emphasized etiology and therapy [see Masserman (5) and Liddell (6, 7)]. Substantial progress, however, has been made in the analysis of the experimentally induced neurotic manifestations by Gantt (8), Anderson & Parmenter (9), and Minami, Moore, & Liddell (10). Babkin (11), Liddell (12, 13), Masserman (5, 14), and Hebb (15) furnish comprehensive reviews and theoretical discussions concerning the present status of this field of research.

Before detailing the recent findings just mentioned, consideration should be given to a question which has been repeatedly asked but not satisfactorily answered. What is the nature of the interrelationship of the facts concerning animal neurosis and the facts concerning human mental disorder?

It is interesting to contrast two statements from the last writings of Freud and Pavlov, concerning the biology of mental disorder. Freud [(16), p. 118] says:

"Man seems to be the only animal with a latency period and delayed sexuality. Investigations of primates, which so far as I know have not been made, would furnish an invaluable test for this theory. It must be significant psychologically that the period of infantile amnesia coincides with this early blossoming of sexuality. Perhaps this state of affairs is a necessary condition for the existence of neurosis, which seems to be a human privilege, and which in this light appears to be a survival from primeval times—like certain parts of our body."

Pavlov [(2), p. 179], in more dogmatic vein, writes:

"The most manifest evidence of the fact that the study of conditioned reflexes has directed us along the right course in investigating the higher parts of the cerebrum and that, in this way, the cerebral functions and the phenomena of our own subjective world have been at last joined together and identified has been obtained from further experiments with conditioned reflexes carried out on animals. They have succeeded in reproducing certain pathological conditions affecting the human nervous system—such as neuroses and certain psychotic symptoms—with the result that in many of the cases it was found possible to achieve a rational and intentional return to the normal standards, or in other words, to effect a cure—a genuine scientific mastering of the subject. . . . The hard conditions which chronically disturb nervous equilibrium consist in the overstraining of the excitatory process, in the overstraining of the inhibitory process and in the direct col-

lision of both these opposed processes, or in other words, in the overstraining of the mobility of these processes."

As might be expected, the answers to our question range between the extreme views just quoted. Some examples will serve to illustrate present diversity of opinion concerning the significance of experimental neurosis. Diethelm (17) believes that what Pavlov described under the misleading term of neurosis corresponds to tension in man, and that just as in patients where pathological tension takes a long time to disappear, so in cases of experimental neurosis the tension will not disappear unless the stimuli associated with the difficult conditioning are removed or avoided. Such animal experiments, he believes, only amplify and corroborate but can never take the place of experiments on human beings in psychobiological research. Kubie (18, 19) similarly holds that "experimental neurosis" is not a true neurosis in the human sense, but is a preneurotic affective disturbance reflecting repeated delays or frustrations in the satisfaction of recurrent needs for food, escape, etc.

Hebb (15), who believes that in human neurosis the condition is simultaneously generalized and persistent after the cessation of the exciting cause, fails to find evidence of the analogue of this condition in the reports of experimental neurosis in dog, cat, or sheep. He grants that the symptoms described in these animals may parallel those of human anxiety states, but that it is anxiety arising only when there is cause to anticipate a recurring injury, not a generalized anxiety, nor one persisting after the precipitating cause has ceased. Neither in animals nor man is dislike of a particular thing or place, avoidance of persons or thing associated with unpleasant events, or temper tantrums to be taken as evidence of neurosis. He finds no conclusive evidence in the experimental animals just mentioned of "endogenous" anxiety, two forms of which are recognized that occurring spontaneously, and that, which although originally "exogenous" continues long after termination of the precipitating noxious events. It is this "endogenous" anxiety which is neurotic. Hebb has found evidence (reviewed on p. 574) of it in two of his chimpanzees but he is not convinced that in the other mammals subjected to experimental procedures there is satisfactory evidence of a psychopathological state, i.e., breakdown of the emotional mechanism as Gantt, Masserman, and Liddell believe.

Grinker, on the basis of his studies of war neurosis (20, 21), believes that, although one cannot make diagnoses in the case of the experimentally neurotic animals in terms of what the human clinical picture shows, it may prove useful to think of the war neuroses as experimental neuroses (22). For example, some patients may show no evidences of subjective anxiety but exhibit tachycardia, increased perspiration, and startle reaction, much as may be observed in some neurotic animals, so that the neurotic reaction in both animal and man may be thought of as due to the establishment of some fixed pattern in the visceral nervous system which is self-perpetuating. "It is," says Grinker, "as if the diencephalic waves of neural energy continued beating in closed circuits of internuncial neurons, maintaining excitation for weeks or months" [(21), p. 131]. Thus neurotic behavior may be entirely internalized.

It is interesting to note that Kubie (23) in discussing the emergency treatment for acute war neuroses reviews deconditioning procedures similar to those which Masserman (5) has successfully employed in alleviating the symptoms of experimental neurosis in his cats.

For example, troops in training have been exposed to "live bullets" and shrapnel-free land mines. In a Southern Pacific "Battle Noise School" men suffering from war neurosis have been exposed in a carefully regulated manner to mock strafing, land mine explosions, and simulated dive bombing attacks while protected from the possibility of physical injury.

Another view of the relation of experimental neurosis to neurosis as observed in the human subject may be stated as follows: The experimental neurosis, which may be precipitated by difficult conditioning or equivalent training procedures, is a primitive, relatively undifferentiated behavioral disorder not to be identified with any clinically defined mental illness, either neurosis or psychosis. It may be regarded as the anlage or prototype of the elaborately differentiated forms of human behavioral abnormality. Like them, however, it may be self-perpetuating and persist until death. Corresponding to the morphologist's conception of a body plan one may think of a mammalian behavioral plan, a pattern of behavior sufficiently general to embrace all mammals including sheep, goat, and man. Pavlov's classical conditioning method can be employed as a traumatizing procedure and, as a method of

training, can exert upon the mammal's nervous system effects essentially similar to the traumatic influence of certain cultural factors in human life. These traumatic effects act through progressive restriction of the animal's freedom both in space and time. It finds itself, as the result of this training, in a psychical strait jacket which prevents it from evading the difficult decisions required of it by the experimenter. The experimental neurosis may be caused by the equivalent of a human conflict situation. The neurosis itself expresses the chronic distortion of the animal's basic behavioral pattern which distortion can be experimentally analyzed, not only as regards functions of skeletal muscle, heart, bladder, etc., but also in terms of altered adjustment to the experimenter and to other animals [Liddell (6, 22)].

Finally, Masserman (14) has integrated his thoroughly systematic extensive observations of experimental neurosis in the cat with his psychiatric studies into a general biodynamic theory of behavior. He has formulated principles of motivation, of experimental interpretation and adaptation, of deviation and substitution, and of conflict which he expounds by means of experimental illustrations drawn from cat behavior and clinical examples. He then makes specific applications of these principles to the field of psychiatry.

#### ETIOLOGY

In a recent symposium on the significance of experimental neurosis, under the auspices of the American Society for Research in Psychosomatic Problems (22), age as a possible factor in the etiology of animal neurosis is considered. Hoskins notes that the maturity process is less securely anchored in man than in other mammals and that man is the only animal capable of developing a clean-cut, definite schizophrenic reaction. [See also Hoskins (24).] Hence, the investigator of animal neurosis might more closely approach the clinical manifestations of neurosis if quite young animals were selected for experimentation rather than the adult animals. Kennard, observing infant monkeys removed from their mothers during the first week of life and kept routinely for about three months, found that certain neurotic potentialities came out markedly. A number of them were thumb suckers and a few could not learn to suck the nursing bottle and had to be returned to their mothers. She believes, consequently, that one must go back to very

early months, if not days, in the life of experimental subjects if one is to prognosticate which individuals will most readily develop experimental neurosis.

Grinker (20) finds that combat stress can be shown in many cases to have reactivated neurotic patterns established in the patients' early years. Hebb (15) reports spontaneously occurring episodes of abnormal behavior in two female chimpanzees. In one, aversion to and even marked fear of large pieces of food suddenly developed and then unexpectedly disappeared to be replaced by hostility toward the experimenter with whom the animal had formerly been on friendly terms. The other chimpanzee whose previous behavior had been noted as unpredictable exhibited, from time to time, unexplained episodes suggesting deep depression. She would appear to be fairly well adjusted for some months and then go into a profound depression without intermission for as long as eight months and during this period would exhibit extreme lack of responsiveness and of spontaneous activity. She would sit for hours with her back to the wall staring at the floor and not looking up when spoken to although, ordinarily, people were her main interest. During feeding times she was similarly unresponsive and had to be caged alone to enable her to get enough food. Even under the carefully controlled living conditions in the Orange Park colony the case histories of these two chimpanzees failed to suggest specific environmental factors to account for their sudden and prolonged disorders of behavior.

The simplest and easiest method for precipitating experimental neurosis in larger mammals has been employed by Masserman (5) for more than ten years in his investigation of abnormal behavior in the cat. The animal is trained in an automatic experimental cage to lift the lid of a box in response to a bell-light signal and secure a pellet of food. When it has formed this simple habit, it receives a blast of air in the face at the moment of taking the food. This fear arousing experience, repeated on two to seven occasions at irregular intervals over a period of a few days, precipitates a chronic experimental neurosis which is described by Masserman [(25), p. 637] as follows:

"somatic and visceral manifestations of anxiety in and out of the experimental situation, frequent startle reactions with specific 'phobic' hypersensitivity to feeding signals and to confinement in small spaces, typical 'compulsive' patterns of hiding or escape, motor disturbances ranging from diffuse restlessness to catalep-

toid immobility, 'regressive' automatisms such as excessive licking or preening, and marked behavioral inhibitions which, when they concerned food, resulted in self-starvation to the point of severe cachexia."

These behavioral aberrations he believes to be comparable to the symptoms of human neuroses.

More complex habit patterns may be built up prior to the onset of the neurosis, such as the operation of a pedal switch giving the signal and releasing the food; or the cat must learn to pass a barrier in order to reach the food box. He attributes the neurosis to severe conflict of motivations, hunger versus fear. This self-starvation due to the frightening experience with the air blast increases the severity of the conflict and worsens the neurosis.

Sutherland, in unpublished experiments described in Liddell's review (13), employed another type of conflict situation to precipitate experimental neurosis in the pig. The animal, habituated to a restraining harness similar to that used by Pavlov in his conditioned reflex experiments (often called the Pavlov frame), learned to lift the cover of a box at a signal to secure food on one day. But on the next day the same signal was followed by an electric shock on the foreleg. The temporal pattern of signalling was identical for the two days. Thus, by alternating feeding and shocking days, the pig was subjected to conflict concerning its interpretation of the familiar laboratory room. On one day it was a place to be fed, but on the next day it was a place of punishment. The animal could maintain its equipoise only when the meaning of the laboratory environment remained stable, either rewarding or threatening. The animal could not sustain successively conflicting interpretations attaching to the same locale.

Another useful procedure for precipitating chronic neurotic agitation has been extensively employed in Liddell's laboratory. [See Anderson & Parmenter (9).] It, also, is based upon the animal's conflicting interpretations of its surroundings. In this case a signal of approaching electric shock on the foreleg is followed by a signal meaning no shock. These positive and negative signals, each of ten seconds' duration, are alternately given and are spaced by constant pauses of five to seven minutes between signals. For example, a telegraph sounder clicking once a second means shock but twice a second means no shock. The animal, restrained in the Pavlov frame, is alerted every seven minutes, first, by a clicking sound for ten seconds which threatens shock, then, after seven



minutes, by a clicking sound again for ten seconds which promises immunity from shock; and this alternation is twice repeated each day. Since the temporal pattern of signals never varies, the animal is compelled to maintain vigilance during the whole hour of the daily test and to undergo rigidly timed cycles of recurring anxiety.

A still simpler procedure for arousing recurring anxiety in sheep and goat, under the condition of self-imposed spatial constriction in the Pavlov frame was first noticed by Anderson (9) and has subsequently been confirmed in our laboratory. Negative conditioned stimuli, i.e., signals meaning immunity from the electric shock, are not employed. The rigid temporal pattern to which the animal is subjected consists only of signals for shock ten seconds in duration separated in our present experiments on sheep and goats by pauses of six minutes. Since the clicking telegraph sounder always means an impending electric shock, the simplest possible pattern of monotonously recurring tension is aroused day after day until the experimental neurosis commonly manifested in the sheep by agitation develops as described in detail by Anderson & Parmenter (9).

In recent experiments Liddell (6) and Minami, Moore & Liddell (10) have demonstrated a new type of experimental neurosis in the goat resulting from a simple modification of the motor conditioning procedure just described. The ten-second signals for shock are separated by pauses of two minutes instead of six minutes and twenty signals per day are given. In sharp contrast to the agitation which results from spacing the shock signals six minutes apart, the shorter pauses between recurring signals for shock lead gradually to a chronic state of tonic immobility. In this condition, there is a strong resemblance to conversion hysteria. The foreleg in response to the signal is rigidly extended rather than freely flexed, while the heart rate shows little or no increase before the shock. At the end of the tests the goat may limp from the laboratory, on the stiffened forelimb, but runs freely in the pasture. As in the case of the agitated type of experimental neurosis, the animal avoids the experimenter and laboratory attendant.

It now seems probable that one can select at random a sheep or goat and confidently predict the type of experimental neurosis that will develop when it is subjected to rigid temporal conditioning. With six-minute pauses between signals the result will be agita-

tion, whereas the animal will come to exhibit the type of experimental neurosis characterized by tonic immobility when the pauses between shock signals are but two minutes.

#### FURTHER MANIFESTATIONS OF EXPERIMENTAL NEUROSIS

Gantt (8) reports the results of the most thorough and long-continued study of a single case history in the literature on experimental neurosis. The dog Nick was the subject of continuous observation and experiment for twelve years with about fifteen thousand conditioned reflexes separately recorded. He exhibited various manifestations of experimental neurosis for a decade. Of these manifestations, the following are of particular interest because they would have escaped observation if the study of this dog had been limited to a period even as long as two years. Nearly three years after the difficult training in the discrimination of tones which led to the onset of the experimental neurosis had been discontinued, Nick began frequent micturition in the experimental room, sometimes as often as once a minute. Punishment was without effect and he began to urinate in the elevator and corridors on the way to the laboratory. About two years following the onset of the pollakiuria, abnormal sexual erections were elicited by the environment of conflict. At first infrequently and then oftener, the abnormal sexual excitations occurred upon entering the conditioning laboratory, in response to a conditioned stimulus, or in the presence of the experimenter even when Nick met him on a farm away from the laboratory. These abnormal sexual manifestations were experimentally analyzed.

The spread of the neurotic disturbance in the course of years to involve chronically many physiological systems is scheduled by Gantt [(8), p. 134]:

"At first there were general definite changes in activity, extreme restlessness, inhibition of the food conditioned reflexes (as early as 1933); later appeared stereotyped respiration, the pollakiuria (1935, 1936) and finally in 1937 the pathological sexual erections—first recorded on 20 May, 1937, to the sound of the metronome."

Through the prolonged study of Nick's case, Gantt was enabled to investigate the changing social and personal relationships of this experimentally neurotic animal. In so doing, he was led to change his point of view. In the foreword to his monograph he says:

"Originally I had felt that more research on the physiology of the conditional

reflexes was in order before a successful discussion of the pathology could be completed. . . . But though important gaps still exist in the normal physiology, I have become more aware of the impossibility of adequately describing normal and abnormal behavior except in the terms of the elements of behavior, notwithstanding the important biochemical, physical, and physiological correlations that should be made at every step."

Liddell (6) records a similar change in point of view during a long continued study of experimental neurosis in the sheep. In brief, the effect of social and personal relationships upon Nick's neurosis were as follows: The manifestations of his neurotic condition in the laboratory were alleviated by the presence of any person standing by him during the conditioning tests. The training tone, formerly a signal for food, caused whining, trembling, drawing away from the food box and, during later years, sexual excitation. Any individual, even the experimenter toward whom Nick was hostile, proved reassuring, and these manifestations of anxiety were absent or much diminished in intensity. Another dog in the room with Nick had no effect upon his anxious behavior, although a bitch in estrus either kept in the paddock with him, or in the experimental room during the tests, alleviated his neurotic symptoms.

Experimental neurosis also affects the behavior of an animal in its competition with other animals in a controlled situation. Masserman (25) records results of a recent study of feeding competition among cats trained by pairs in a conditioning cage. Food could be secured by opening the cover of a box at a bell-light signal. It could also be secured as often as desired by depressing a platform switch which automatically activated the signal and feeder mechanism.

In the first situation, when the experimenter gave the signal the dominant cat secured the food without contest; the submissive animal ignored the food box, backed away from it, or explored the cage. Following the onset of neurosis the dominant cat now reacted phobically to the signal while its partner fed, but then the neurotic animal would attack its successful rival.

In the second situation, one cat usually became a worker, the other a parasite. The worker would develop techniques for depressing the platform switch a number of times in rapid succession so that enough pellets were delivered to enable it to reach the container before the parasitic cat could consume all the food. A

worker cat, after the onset of neurosis, avoided the switch and so made it necessary for its parasitic partner to operate the switch in order to get food.

Two frames of reference, biological and psychological, must be employed in appraising the significance of the experimental neurosis. Perhaps at present the psychological framework is the more useful to the investigator. With the experimental animal the investigator cannot communicate as the physician does with patient. To balance this lack, however, the psychological setting in which the animal's behavior occurs is enormously simplified. In the laboratory the experimenter can fabricate and largely control the stresses to which the animal subject is exposed during its regimen of training. He can drastically simplify the psychological environment both by choosing mammals whose behavior is less complex than that of man, or even the chimpanzee, and by bringing as many as possible of the simple variables in the laboratory situation under control. From the study of such a skeletal psychological environment it will be possible to identify the traumatic factors responsible both for animal and human neurotic behavior. Such an analysis will contribute fundamentally to psychotherapy.

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DEPARTMENT OF PSYCHOLOGY  
CORNELL UNIVERSITY  
ITHACA, NEW YORK

## PERMEABILITY<sup>1</sup>

BY W. WILBRANDT

*Pharmakologisches Institut, University of Berne, Switzerland*

Several surveys on permeability have appeared, the most extensive one, that of Höber (1), covers the entire field including particularly work on accumulation and active transfer. This phase of permeability is also the subject of a review by Krogh (2). Danielli & Stock (3) have surveyed the permeability of blood capillaries, and Hoagland (4), the uptake and transport of ions by plant roots. Collander (5) has tabulated quantitative data on electrolyte permeability and salt accumulation in plants (appeared in 1942, written in 1938). A monograph of Bürgi (6) treats the permeability of the skin for drugs and poisons. A review by Landis (7) contains data on capillary permeability. Wilbrandt (8) has tabulated data on permeability of animal cells (also written in 1938). Danielli's contributions to Bournes' "Cytology and Cell-physiology" deal with related subjects (9, 10). Additional reviews treat capillary and cell permeability (11), comparative permeability studies (12), the "permeability vitamine" P (13), and cell permeability and diffusion (14). Some thirty papers dealing with hemolysis and other problems related to the structure of the cell membrane had unfortunately to be omitted for lack of space.

Work on passive permeability as well as on accumulation processes and active transfer, which are being studied with increasing interest and success, will be included in this review. Since, however, the border line between these two fields has not yet been precisely defined, the data will be grouped primarily according to the substances involved rather than to the mechanism. Biopotentials will be classed under ion permeability.

### PERMEABILITY TO UNDISSOCIATED MOLECULES INCLUDING WATER

*Erythrocytes.*—An interesting extension of the species specificities in permeability discovered by Jacobs is the finding that the

<sup>1</sup> This review covers the period from July, 1944 to July 1, 1946, the period since the last review by Brooks in Volume VII, 1945, although some earlier papers not previously reviewed have been included. Many foreign journals from this period, particularly American, are still lacking in Switzerland, and in spite of the generous supply of reprints from various workers, there remain gaps, which, it is hoped, will be filled out by the next reviewer.

apparent permeability to thiourea differs significantly between red cells of white and negro subjects (15). Ørskov (16) extended his studies of permeability changes *in vivo* under the influence of hemopoietic substances and found similar effects with various extracts including those from yeast and meat and several vitamins. A minimum value of the red cell permeability for oxygen is given by Roughton (17) from experiments with his rapid reaction method. Goevaerts & Lambrechts (18) studied the water permeability using heavy water. Temperature coefficients of permeability for water and seven compounds were determined on red cells of turtle, chicken, and four fish species by Hunter (19). Hunter & Stringer (20) report that treatment with heat decreases the lipid content of the chick erythrocyte membrane and increases the rate of hemolysis in glycerol solution. Ballentine (21) finds no appreciable species differences in the stromatin composition with regard to leucine and glycine, which together with the corresponding results of Dziemian concerning lipid would seem to indicate that species specific permeability depends on structural arrangement rather than on the chemical composition of the membrane.

*Cells of various animal organs.*—From *in vivo* experiments with radio-phosphate, Sacks (22) concludes that the entrance of glucose into the muscle is preceded by phosphorylation and splitting of the ester on the cell membrane, leaving phosphate behind. Fishler *et al.* (23), also using radio-phosphate, found phospholipid formation in hepatectomized animals in kidney and small intestine, without appreciable increase in plasma phosphatide. In contrast to the liver cells, the cells of these organs, therefore, seem to be rather impermeable to the lipid. Hahn (24) discusses taste sensation in relation to permeability.

*Microorganisms and yeast cells.*—Yegian & Budd (25) find *Mycobacterium tuberculosis* and *M. ranae* permeable for various sulfonamides. Drug-fastness seems not to be due to impermeability. Using a fluorescence method based on suppression of the fluorescence of acridine orange by prontosil, Strugger (26) studied the entrance of prontosil into various microorganisms and yeast cells. Ørskov (27) finds that penetration into yeast cells can be followed by opacimetry. The rate in general seems to depend on molecular volume rather than on lipid solubility.

*Higher plant cells.*—Wartiovaara (28) finds temperature coefficients for the penetration of eight substances into cells of Toly-



pellopsis mostly between 2.5 and 6 (hexamethylenetetramine 9.2). The data show no correlation to the rate of permeation. His experiments on the same cells with heavy water and eight solutes (29) yield permeation constants for the small molecules of water and methyl alcohol, which, compared to their ether solubility, are strikingly high. According to Ruge (30) the epidermis cells of Rhoeo during the course of differentiation become more permeable for erythritol and glycerol, less permeable for urea, methylurea, thiourea, and acetamide. Moser (31) studied plasmolysis of *Cladophora fracta* cells in solutions of glucose, calcium chloride, and potassium chloride and measured permeability for several organic molecules.

*Placenta.*—The data in this and subsequent sections concern the permeability of cell layers rather than cell membranes of single cells. In numerous papers the penetration through the placental wall has been calculated either from determinations of concentration in the umbilical cord blood or from the action of the substance involved on the fetal organisms after application on the maternal side. Thus the placental wall has been shown to allow the passage of vitamins A (32), B<sub>1</sub> (33), and E (34), testosterone (35), penicillin (36, 37), streptomycin (38), not that of neo-arsphenamine (39), that of alloxan (40), only in amounts insufficient to produce permanent diabetes in rats, antipyrine (41).

*Pleura and lungs.*—Bertoli (42) followed the passage of glucose from the pleural cavity into the blood and vice versa on rabbits. Experiments of Roughton *et al.* (43, 44) may lead to a reconsideration of the question of active oxygen transport in the lungs. According to this investigator during exercise at low oxygen partial pressure a diffusion constant of about 200 would be required to meet the demands of transport by diffusion. The maximum values found by Krogh were 60 to 70, and in new experiments the constants do not change under low oxygen pressure.

*Kidney.*—Only the presumably passive movements will be dealt with here. From determination of the urea clearance in man and dog, Dole (45) estimates the permeability of the cells in the distal tubules to be about 1,000 times lower than in erythrocytes. Whereas sulphanilamide has a clearance of only about 30 to 40 per cent that of inuline clearance, according to Loomis *et al.* (46, 47), acetylsulphanilamide yields the same clearance value as inuline, acetylation thus serving to render the drug nonpenetrating and

easily excretable [as "detoxification reactions" may also do in other cases (cf. 1)].

*Mammary gland and lacrimary gland.*—Thyropotamine (48) does not pass the mammary gland of guinea pig and man, whereas in rats dicumarol does. It produces hypoprothrombinemia in the suckling animals (49). In addition DDT appears in the milk of rats and goats (50), its toxic effects being observable even after two passages of the mammary gland. The lacrimary gland of man is permeable to various sulphonamides (51).

*Aqueous humour and cerebrospinal fluid.*—Various sulphonamides were shown to pass from the blood into the aqueous humour (52, 53). The old observation made by Goldmann in 1913 that Trypan Blue, intravenously applied, does not stain the brain tissue in contrast to other organs was extended by Lundquist (54) to four species of fresh water teleosts, so that the low permeability of the blood-brain barrier seems to be a rather common phenomenon. It can be increased by the action of injurious agents like hypertonic salt solutions, acid solutions below pH 4, bile acid, ethyl alcohol, cobra venom, and bee venom (55). Acute or chronic aniline intoxication also injures the barrier (56).

*Blood capillaries in general.*—The permeability of blood capillaries has attracted considerable attention in its relation to various forms of shock and because of the alleged action of certain hormones and vitamins. The methods used depend mainly on the observation of rate of water filtration and of exchange of solutes, particularly radio isotopes, and colloids (Trypan Blue, protein, phosphatides).

Hyman & Chambers (57) devised a method of following the weight changes of perfused organs due to the passage of water to the capillary wall by weighing the organ. They reported changes under the influence of adrenal cortex hormones. Hyman (58) studied the relations of capillary filtration to filtration pressure and colloid osmotic pressure as well as the action of various plasma substitutes. The filtration rate was found to be a linear function of perfusion pressure, while the dependance on colloid osmotic pressure was not linear with all colloids used. Palmer & Joseph (59), using the same method, report experiments on the action of lemon extracts (vitamine P) and adrenal cortex, both apparently yielding only restrictedly satisfactory results. Mylon *et al.* (60) conclude from hemoconcentration after infusion of phosphate and citrate

that calcium increases permeability by an effect on the intercellular cement substances.

In the following list of papers dealing with the exchange of solutes through the capillary wall, in addition to a study on the disappearance of intravenously injected galactose (61), those using radio-ions will be included (62, 63, 64) since the electric charge presumably does not influence the results. Potassium leaves the capillaries faster than sodium (62) due mainly to its higher distribution volume and greater permeability. Heavy water reaches a distribution volume of 34 per cent of the body weight in twenty-one seconds (62). Compared to the sodium data its rate of exchange is found twice that expected (63). Experiments with intravenously injected sodium indicate faster disappearance in liver, intestine, and lungs than in brain, bones, skin, and tendon (64). Joseph *et al.* at short intervals (65) followed pH changes in synovial fluid and blood under varied conditions, e.g., alcalinization by sodium carbonate or acidification by exercise. Curves for the former showed considerable lag and flattened maxima and minima. In from three to six hours, 9 to 20 per cent of intravenously injected radio-phospholipids appeared in the thoracic lymph, indicating considerable capillary permeability for lipoids (66). Sturm (67) describes localized passage of dyestuffs, presumably intercellular, and doubts the existence of cement substances. Danielli (3) points out that the observations underlying the Rous concept of a gradient of capillary permeability are logical consequences of the Starling mechanism on account of water reabsorption.

Clinical investigations (68) indicate unimpaired capillary permeability in cardiac failure as compared to venous congestion in normal persons. Faster entrance of fluoresceine into the skin in myxedema (69, 70) is considered as evidence of higher capillary permeability. Fleckenstein (71) considers metabolic disturbances rather than changes in capillary permeability responsible for the action of allyl formiate. Ponder & Hyman (72) studied saponin edema (for method cf. 57) in relation to the concentration and the uptake of saponin and found the increase of filtration rate to be a linear function of saponin concentration.

The action of histamine was dealt with in several papers. Its local action (entrance of intravenously injected Trypan Blue into the skin) was used to demonstrate the antihistamine activity of RP 2786 (Neo-Antergan) (73), similarly the corresponding action

of ascites fluid was studied (74). In man also intra-arterial application was shown to increase capillary permeability (75), whereas systematic administration failed to do so (76, 77). Roller (78) claims to have found substances increasing erythrocyte permeability in the serum during histamine shock.

Numerous papers deal with the changes during various forms of shock. In traumatic shock, capillary permeability increased near the site of trauma (79). The increase was inhibited by local sympathectomy (80). Contradicting are the results concerning general, rather than local, alteration of capillary permeability in shock. Thus, in traumatic shock protein does not pass (81), but in burn shock (82 to 85) it passes generally into lymph as tested immunologically. Possibly not all methods are equally sensitive or not all colloids equally concerned.

French authors consider epicatechol rather than hesperidin to be most active as permeability-vitamine P (86 to 90, 13). The evidence for its action on capillary permeability is meagre (cf. 59), it is tested mainly by capillary fragility (88, 89) the relation of which to permeability still seems unsettled. In this and other respects it is shown to enhance the action of epinephrine (cf. 13).

The supposed action of hyaluronidase on capillary permeability was tested by Danielli (3) with negative results. Its influence on spreading in the skin was found to be antagonised by morphine (91) and to antagonise that of epinephrine (92).

*Action of detergents and wetting agents.*—Detergents were shown considerably to increase the rate of passage through cell layers: of epinephrine through the cornea (93), of hexylresorcinol in *Ascaris lumbricoides* (94), and pig round worms (95).

*Body surface of whole animals.*—Pyrethrum passes into mosquitoes through the tracheal wall (96). Lipoid solubility seems to be the dominating factor for the passage of organic molecules into the larvae of mosquitoes (97), as shown by comparison of over one hundred compounds. Also the penetration of pyrethrum seems to depend on lipoid solubility (98), as evidenced by parallel changes in toxicity and fat content of the exoskeleton of *Loxostege sticticalis*. The rate of passage of organic and inorganic arsenous compounds in marine fish increases with dilution of the sea water (99).

*Influence of drugs and hormones on cell permeability.*—Bohlmann (100) reports increased rate of hemolysis in water after treatment of the red cells with tannic acid. Antipyretics (101) retard the penetration of urea into cartilage cells, narcotics in low con-

centration that of salts and dyestuffs into cells of *Elodea canadensis* (102). No increased permeability of muscle to radio-phosphate occurs under the influence of insulin (103), but 20-methyl-cholanthrene changes the permeability of epithelial cells in mice (104).

*The mechanism of drug action in relation to permeability.*—Macovski (105, 106) treats the relations of toxicity to dosage both mathematically and experimentally by assuming a threshold concentration and transport by diffusion. Genuit *et al.* (107, 108, 109) deal critically with the assumption made by German workers that acetylcholine acts according to its concentration gradient rather than to its concentration and that atropine acts by preventing acetylcholine from entering the cells. Certain features of acetylcholine action invoked as support of the theory such as the latent period and saturation dose, are also observed with other drugs including papaverine. Moratschek (110) and Voigt (111), in the same argument, show that mecholyl, esmodil, and choline have no limited time of action, as would be demanded by the "potential-gift" theory. Hurst (112) discusses permeability and molecular constitution with respect to the action of drugs, mainly insecticides.

*Distribution experiments, fractions of body water.*—Thiourea as an indicator of total body water was studied by Danowski (113) who reports reasonable agreement with metabolism determinations and by Williams & Kay (114), who found poor correlation. For extracellular water Kruhffer (115), comparing inulin and thiocyanate, found distribution volumes of 20 per cent and 30 per cent of the body weight respectively. From the different rates of distribution for inulin and saccharose in nephrectomized rabbits, he concludes diffusion rather than convection to be dominating (116). Winkler *et al.* (117) found distribution volumes for thiocyanate, radio-sodium and radio-chloride of 36, 28, and 25 per cent of the body weight respectively. Changes of extracellular water, however, rather than absolute values, agreed well. On *Mytilus*, Krogh (118) finds thiocyanate unsuitable, being distributed in the total water, and suggests the use of thiosulphate. Sulfanilamide is distributed in the total body water according to Alexander (119).

*Permeability of artificial membranes.*—Weatherby (120, 121) prepared collodion membranes containing lipoids, through which salicylic acid and nicotine penetrated best as undissociated molecules (120), and which he suggests as models for the blood-brain barrier (121). Dubouloz *et al.* (122) describe a method for diffusion experiments through collodion membranes and point out that the

customary use of filtration rates for measuring permeability is bound to give divergent results, with pores of radius  $r$  depending on  $\sum r^2$  (Poiseuille) rather than on  $\sum r$  (diffusion). Glückauf (123) concludes from experiments with adsorbing membranes that graded states of adsorption with graded mobilities of the adsorbed molecules must be considered.

*Active transport through cell layers of intestine and kidney.*—Cori's statement that the rate of glucose absorption from the intestine is independent of the amount of sugar is not confirmed for some conditions (124). Hydrogen sulfide inhibits the glucose absorption rate as well as phosphorylation *in vitro*, whereas xylose absorption is not influenced (125). Adrenalectomy inhibits intestinal fat absorption only with higher fatty acids, beginning at caprylic acid (126). Schou (127) finds that infusing large amounts of hypertonic sulfate solutions depresses water reabsorption in the kidney almost completely, two-thirds of the glomerular filtrate appearing in the urine and urine flow rising from 100 to 150 times normal. Eiler *et al.* (128) report inhibition of diodrast secretion in the kidney tubules by phlorizine.

#### PERMEABILITY TO WEAK ELECTROLYTES, AMINO ACIDS, AND DYESTUFFS

*Weak electrolytes.*—The observation frequently made that weak electrolytes penetrate chiefly as undissociated molecules reappears in papers by Cogan & Hirsch (129) concerning the cornea *in vitro* and *in vivo* (aniline, salicylic acid, and others), by Volynskii (130) concerning the frog skin, and by Oivin (131) concerning the skin (hydrogen sulfide). A more detailed analysis of the absorption of fatty acid from the rumen of ewes using Danielli's criteria, revealed at pH 7.5 absorption as anions through water-filled pores (presumably intercellular cement), at pH 5.8 in addition absorption as molecules, mainly through a homogenous lipid layer (132). Accordingly out of a mixture of acetate, propionate, and butyrate at pH 7.5 acetate is absorbed best at pH 5.8 butyrate. Almasy & Fischer (133, 134) studied the inhibition of respiration of *Bacterium soor* by quinine under varied pH. They showed theoretically that the distribution is independent of whether the membrane is permeable for the molecules, the monovalent or the bivalent cations and that the bivalent cation seems to be responsible for the inhibition (inhibition versus concentration in the

cell proving to be independent of pH for this ion). The rate of quinine penetration into amoeba was studied by Rollé (135).

*Amino acids.*—Ussing (136, 137) finds erythrocytes permeable for tyrosine, alanine, and phenylalanine, impermeable for glutamic and aspartic acid. The earlier observed surplus of amino nitrogen in the cells seems to be due to glutathione. The same is made probable for liver, kidney, muscle, and brain. Arisz (138, 139) offers evidence for an active transport of various amino acids and other nitrogen containing compounds through the tentacles of *Drosera capensis*: dependence on oxygen, inhibition by narcotics and cyanide. The renal reabsorption of glycine, alanine, glutamic acid, and arginine is referred by Pitts (140, 141) to a common mechanism, characterized by competition with creatine and limited transport capacity. Similar results are reported by Eaton *et al.* (142) for valine, leucine, and isoleucine. A corresponding mechanism, showing competition with glucose, but no inhibition by phlorizine, is shown for phosphate reabsorption (143). Acidification of urine by reabsorption of phosphate or bicarbonate are ruled out by the same authors (144) showing experimentally that in acidotic dogs the glomerular filtrate contains only 7 to 11 per cent of the phosphate and 16 to 26 per cent of the bicarbonate required.

*Dyestuffs.*—Collander (145) furnished the first quantitative data on the permeability of a basic dyestuff, neutral red. It diffuses 140, 800, and 20,000 times faster than urea into cells of *Chara*, *Tolypellopsis*, and *Allium* respectively and is 10,000 times more lipid soluble. Bazin (146) shows that the rate of penetration of neutral red and Bismarck brown into *Elodea* cells depends on the anion of the dye base. The permeability of frog epithelium cells for nine dyestuffs was reversibly lowered by protein denaturing agents (147). Friedemann (148) reports selective uptake of aniline dyes injected intravenously at the inner surface of the abdominal wall in guinea pigs.

Active transfer of dyes through tubular epithelia in tissue cultures is stimulated by adrenal cortical compounds (149). Haywood *et al.* (150) describe accumulation up to three thousandfold of dyes in the bile of both fresh water and sea water fish.

#### PERMEABILITY TO IONS

The use of isotopes has led to further considerable progress particularly concerning active transport and accumulation proc-



esses. The important problem as to whether the general prevalence of potassium in living cells is due to impermeability to sodium or to active processes keeping it out, has been extensively studied and discussed, the main opponents being Conway (see below) and Krogh (2). Considering the fact that most experiments showing sodium penetration have been done with low potassium or potassium free solutions, the question may perhaps be raised whether sodium can pass a membrane otherwise impermeable to it, if the membranes potential is raised above a critical value, a concept that might reconcile opposing viewpoints. A surprising neglect of the electrical forces involved is also shown by the custom of calculating "permeability coefficients" for electrolytes or ions in the same way as for nonelectrolytes, which may lead to serious misinterpretations.

*Muscle.*—Conway's interesting suggestion that ion permeability is controlled by ionic size rather than charge, the membrane being permeable to potassium and chloride as well as to all smaller ions, has given rise to a lively discussion (151). One difficulty, realized in 1941 by Conway *et al.* (152) and emphasized by Fenn *et al.* (153), is the distribution of ammonium, which should equal that of potassium, whereas Fenn *et al.* find a maximal inside to outside ratio of only one to five after several days. Conway & Moore (154) report similar results with outside ammonia concentrations higher than 1 mg. per cent nitrogen; at lower concentrations, however, they find higher ratios reaching the theoretical value, as well as an increased rate of ammonia entrance under higher pressures of carbon dioxide and impermeability in absence of bicarbonate. They conclude normal impermeability for ammonium and a permeability change in presence of carbon dioxide, but there remain questions which recall the related controversy between Jacobs and Ørskov concerning erythrocytes. The argument of Wallace and co-workers (155, 156) that the bicarbonate to hydrogen ion distribution ratio is too high is met by Conway & Fearon (157) with the claim of a barium soluble fraction of acid labile carbon dioxide, which gives rise to false bicarbonate values. Correction for this unidentified fraction leads to the theoretical ratio for both bicarbonate and hydrogen.

The remark of Höber (1) and of Krogh (2) that the high potassium concentration in Conway's experiments injured the muscles is answered by experiments with only 7.5 m.eq. sodium replaced by

potassium, in which the expected volume change occurs at the same rate (158). Krogh's suggestion of active transport of sodium out of the sodium permeable cells meets with two difficulties (158). Assuming equal permeability as for potassium, it would require an amount of energy equal to twice the resting metabolism. Furthermore the sodium impermeability is not interfered with by cyanide. In accordance with Conway's theory, Wilde (159) finds an increase of fiber chloride in rats *in vivo* after potassium injections. From swelling rates Conway & Moore (160) derive permeability constants for various salts. Steinbach (161) studied the degree of swelling in anisotonic Ringer solutions with constant potassium and calcium and varied sodium. Deviations from the calculated volumes (which however do not seem altogether unreasonable) lead him to the somewhat surprising conclusion that the muscle fiber is permeable for all ions of the external medium.

According to Ferrebee *et al.* (162), desoxycorticosterone, like potassium depletion, causes sodium to replace intracellular potassium. In adrenalectomized rats Jordan (163) finds loss of potassium from active muscles. Quantitatively the rate of loss is about the same as normal, if referred to equal tension time products of the muscle. Verzar (164) finds the rate of swelling of frog muscles in isotonic potassium chloride solutions decreased by adrenalectomy or iodoacetate. The rise of muscle and serum potassium in sodium-depleted rats, originally referred to impaired renal excretion, occurred also in short-time experiments of Miller (165) and occasionally with considerable diuresis, so that extrarenal factors must be considered.

Noonan *et al.* (166) and Lyman (167) find an increased rate of entrance of radio-potassium into denervated or (166) stimulated rat muscle. This is, however, referred by Noonan *et al.* to circulatory rather than to permeability changes, since it fails to occur in isolated frog muscles or frog muscles *in situ*.

*Heart muscle.*—Krogh *et al.* (168) studied frog hearts in potassium-free solution and found exchange of two-thirds of their potassium for sodium. Some of the intracellular potassium is assumed to be bound and evidence for intracellular chloride is given. Replacing four-fifths of the sodium chloride in the Ringer solution by glucose led to loss of intracellular sodium, apparently in exchange for sugar (169).

*Kidney and liver.*—From experiments on kidneys similar to

those on muscles supporting the Conway theory a "standard permeability" is observed (170) for most of the cells; for a small fraction, however, the ability for active extrusion of sodium is indicated by a decrease of the sodium space. From histological observations this latter is identified with the distal, the sodium impermeable fraction with the proximal tubules. Darrow & Engel (171) find loss of potassium in exchange for sodium from the liver in hemorrhagic shock, which they refer to suppressed metabolic activity rather than to structural changes (parallelism to enzymatic disturbances).

*Skin and other epithelial layers.*—Eichelberger *et al.* (172), studying the electrolyte distribution in dog skin find per kg. fat-free tissue 86.7 mM chloride, 96.5 mM sodium and 22.4 mM potassium, which requires the assumption of intracellular sodium as well as chloride. Meyer & Bernfeld (173) studied the ion permeability of the frog skin potentiometrically in terms of the Meyer-Teorell theory of membrane potentials. They found the outer surface cation-permeable in contact with sodium chloride, but anion-permeable when in contact with unbuffered potassium chloride. Evidence is offered for acid production in contact with potassium chloride to account for this reversal of charge. The inner surface was indifferent, a deeper layer (long latency) however reacted to pH changes like a glass electrode. An analysis of Reins earlier data concerning human skin yielded  $A=0.001$ , cationic selectivity (174). Experiments by Krogh (175) on the chorion membrane of the hen's egg revealed (a) loss of potassium, the latter exchanging for sodium in potassium-free solutions and (b) active uptake of potassium in exchange for sodium from low potassium concentrations. The rate of passage of sodium through the human placenta increases during gestation (176). Cohn & Brues (177) describe permeability determinations in tissue cultures using radio-isotopes.

*Erythrocytes.*—The criteria proposed by Wilbrandt characterizing those types of hemolysis which are caused by induced higher cation permeability and consecutive swelling ("colloid osmotic hemolysis") have been applied to further cases. Photodynamic hemolysis was shown to be colloid-osmotic (178), ultrasound hemolysis nonosmotic (179). The temperature coefficient of hemolysis after irradiation may be negative at low temperature, indicating that adsorption processes are involved (180). Compensation of the inside colloid osmotic pressure by nonpenetrating

saccharose outside in colloid osmotic hemolysis experiments (x-ray and ultraviolet hemolysis) leads to predictable equilibrium volumes rather than to hemolysis (181). A curious hemolysis in calcium-free media, specific for blood of some marine fish and of the snapping turtle has been described by Lyman (182) and likewise interpreted as due to salt permeability. Honey bee venom has been shown by Levi (183) to increase cation permeability for radio-potassium. Malinin (184) claims an increase of ion permeability by amboceptor. Radio-lead is shown to penetrate rapidly into dog erythrocytes *in vivo* as well as *in vitro* and to accumulate fortyfold (185). The nuclear membrane of frog erythrocytes is freely permeable to ions (186).

*Plant cells.*—Holm-Jensen *et al.* (187) on the basis of tracer experiments give the following permeation constants in cm. per hr.: in cells of *Tolypellopsis*, potassium  $3 \times 10^{-5}$ , sodium  $1 \times 10^{-5}$ , in *Nitella* cells, potassium  $0.8 \times 10^{-5}$ , sodium  $1 \times 10^{-6}$ . More than 90 per cent of the diffusion resistance lies in the outer membrane. In *Chlorella pyrenoidosa*, Scott (188) finds no loss of any cation into water, no loss of potassium into any solution, but losses of magnesium and calcium into salt solutions in exchange for other cations. Radio-lead is shown to accumulate in protoplasmic granules of *Nitella* previous to accumulation in the sap (189). Potassium in yeast can be completely exchanged for ammonia (190, 191). Biologically (respiration, growth) "ammonia yeast" differs from "potassium yeast" quantitatively, but not qualitatively. Intracellular potassium thus is not essential. By exchange of hydrogen inside for potassium outside yeast cells may produce a pH of 1.78 in unbuffered solutions (192). Hevesy & Zerahn (193) applying high doses of x-ray and ultraviolet rays to yeast cells induced little increase of radio-potassium permeability, but a two- to sevenfold acceleration of radio-phosphate loss, which they refer to as permeability change. Electrolyte permeability changes with aging in cells of onion, cabbage, beans, and oats have been described (194).

From potential measurements in *Nitella* cells, Osterhout (195, 196, 197) concludes that the inner protoplasmic surface may be injured before the outer by formaldehyde and mercuric chloride. An apparent reversal of charge as judged by the sign of the concentration potential is brought about by potassium hydroxide, less so by sodium hydroxide, which Osterhout (198) refers to dissolu-

tion of a membrane constituent, possibly a fatty acid. Guanidine (199) increases the electrical excitability. Osterhout further describes a plastic behaviour of the inner membrane showing changes of shape due to protoplasmic streaming (200).

*Biopotentials in animals.*—Steinbach (201) reports concentration potentials at the injured surface of frog muscle, both with potassium chloride and sodium chloride, reaching 40 to 60 per cent of the theoretical maximum. The aerobic fraction of the resting potential of frog nerve (the fraction recovering in oxygen after nitrogen) depends on potassium concentration in a way similar to oxygen consumption (Shanes, 202). Drawing current decreases the resting potential of nerve by accumulation of a substance, presumably potassium, outside the fibers (203). Hodgkin & Huxley (204) measured action potentials of squid axons of up to 90 mv. with resting potentials of 45 mv. (the true resting potential without short circuiting being estimated as 60 mv.), so that a reversal of the membrane potential must be assumed. Acetylcholine does not depolarise nerve even in nearly isotonic concentration (205). It retards anaerobic depolarisation and accelerates aerobic repolarisation, qualitatively resembling magnesium. A participation of acetylcholine in nerve conduction is considered therefore improbable.

*Artificial membranes.*—Meyer & Bernfeld (206, 207, 208) describe the preparation of basic, neutral, and acid membranes from various materials as well as potentiometric analysis of asymmetric membranes and of mosaic membranes. Sollner *et al.* report standardized methods for the preparation of reproducible collodion membranes of high selectivity and conductivity ("megapermselective"), (209, 210, 211), based on oxidation of the membrane by sodium hydroxide and standardized drying conditions. Positive membranes (coated by protamine) of similar properties can also be prepared (212, 213). Determinations of the number of acidic groups in collodion membranes gave values one to two orders lower than those calculated by the Meyer-Teorell theory. From this and other instructive experiments (214, 215, 215) the Meyer-Teorell theory is inferred to lack an element accounting for structural properties of the membrane. The possibility of using selective membranes for the determination of ion activities is pointed out (217, 218). Wilbrandt treats the kinetics of ion exchange through selective membranes theoretically (219) and in experiments on erythrocytes (220). Austin (221) reports deter-

minations of diffusion rates of salts through the copper ferrocyanide membrane with an improved method.

*Electrical conductivity.*—The high resistance of the human skin to alternating current and its rectifier effect (dependance of direct current resistance on the direction of the current) are due to the stratum corneum, as Rosendal (222, 223) showed. It drops to 0.5 to 1 per cent of its value after elimination of the corneous layer and thus is not due to a polarisation capacity of the epithelial cells. The rectifier effect of the nerve membrane of the squid axon was shown (224, 225) to decrease with increasing potassium concentration outside and to be enhanced by calcium. The impedance of stomach muscle and rectus abdominis in frogs and dogs has been studied (226), as has the increase in impedance during contraction of turtle ventricular muscle (227). Studies on the impedance of frog stomach mucosa indicate a reversible increase of the membrane potential and a rise of the phase angle towards  $90^\circ$  after changing potassium for sodium in the mucosa solution, which is referred to high potassium permeability (228). Parallel changes of polarisation capacity and permeability for urea and sodium chloride were found in the frog skin (229).

*Active ion transport through the intestinal wall.*—Visscher *et al.* published several interesting papers with radio-isotopes showing active transport. In passive diffusion systems from the rate of out diffusion and the concentrations on both sides of the membrane the rate of diffusion into the system and the net result can be calculated. Such calculations for the intestine do not agree with the observation concerning sodium (230), water, and chloride (231), indicating active transport mechanisms. The sodium transport shows a gradient decreasing from the jejunum to the colon and a rate of turnover as high as total plasma sodium in eighty-three minutes. In accordance with the experiments on chloride transport, absorption of autogenous serum occurs with decrease of osmotic activity (232). This decrease, in experiments with salt solutions, is inhibited by mercuric chloride (233). Perryman *et al.* (234) studied the influence of glucose on intestinal phosphate absorption without unequivocal results. Radio-ferrous iron was better absorbed than radio-ferric iron (235).

*Acid secretion in the stomach.*—Rehm published a series of papers showing a stomach resting potential across the mucosa of about 70 to 95 mv., decreasing with the onset of secretion (histamine) (236). An external potential influences the secretion ac-

cording to its sign positively or negatively (237). Thiocyanate inhibits secretion and increases the potential to the resting level, in lower doses the inhibition precedes the potential rise (238). The current output of the stomach mucosa through a low resistance yields a maximal supply of electrical energy sufficient for the production of 200 cc. juice in twenty-four hours (using estimates by Davenport). As much as 68 to 276 microamperes could be continuously drawn (239). Conway *et al.* (240) offer a theory of acid secretion, assuming a primary secretion of approximately isotonic potassium chloride and a secondary exchange of potassium for hydrogen analogous to his aforementioned observation on yeast [(192) (cf. also 228)]. In view of the carbonic anhydrase theory of secretion the finding of Conway & MacDonnell (241) may prove important in that, in yeast, decarboxylation liberates carbonic acid rather than carbon dioxide.

*Active ion transport through other cell layers.*—The lacrimal fluid of man is not hypertonic, as indicated in several Pharmacopoeia, its osmotic equivalent being 0.87 to 0.92 per cent sodium chloride (242). Becker (243) studied the chloride transport through the frog skin. That the chloride ratio of plasma to aqueous humour is 0.93 rather than 0.96 (theoretical Donnan distribution), was confirmed (244). After paracentesis, however, the freshly regenerated humour showed the theoretical ratio, regeneration apparently occurring by filtration, on which secretion processes were superimposed later. Weir (245) injected bromide intracisternally into dogs, previously treated with bromide intravenously, and found the bromide as well as the chloride ratio of plasma to cerebrospinal fluid to approach the theoretical value, which he refers to either inhibition of secretion in the plexus or enhanced filtration through the brain capillaries. Intravenously injected radio-sodium passed into the aqueous humour about twice as fast as into the cerebrospinal fluid, reaching 75 per cent equilibrium in forty-five minutes (246). Krogh (247) found active transport of silver ions in *Eristalis* larvae through filaments hitherto regarded as respiratory organs.

*Ion accumulation and active transport in plant cells.*—Collander (248) reports analyses for cations in root and shoot of sixteen plants grown in salt solutions. Potassium, rubidium, and calcium were found about equally concentrated, whereas sodium and manganese concentrations were mostly higher in the root and calcium, strontium, and lithium concentrations higher in the shoot.



Reilhes (249) studied the penetration of heavy metals into the vacuoles of root cells by microscopic observation. Experiments with radio-rubidium and radio-phosphate revealed accumulation into barley roots from  $10^{-9}$  *M* solutions, maximal at a distance of 1 mm. from the tip, which is not prevented by low temperature (250). Potassium bromide accumulation is accelerated in excised barley roots by calcium, magnesium, strontium, barium, and aluminum, these substances presumably acting on the surface membrane (251). Carbon dioxide inhibited water absorption through roots of wheat, maize, and rice by 14 to 50 per cent (252). Schuffelen & Loosjes (253) calculate the rate of cation absorption from root potentials assuming simultaneous loss of cations proportional to their concentration. Lundegardh, in an extensive paper (254), gives a representation of his work on ion absorption by roots. In new experiments he finds divergent accumulation of potassium and bromide, the former showing maximum concentration at the tip of the root, the latter increasing with the distance from the tip. In a theory of "anion respiration" he links the anion accumulation with the valency change of catalytic iron, arguing that trivalent iron exerts a stronger electrostatic attraction to negative ions than divalent. Ernst (255) refers potassium accumulation to thermosmosis and related phenomena. Cyanide and azide inhibit two-thirds of the respiration and completely suppress bromide accumulation in excised barley roots (256). Partial respiration inhibition by cyanide is accompanied by disproportionately strong inhibition of accumulation, indicating a second action of cyanide. The rate of oxygen uptake and carbon dioxide production in barley roots were found by Machlis (257) to have a rather steep gradient, showing at a distance of 9 cm. from the tip only 16 per cent of the tip value. Potassium chloride 0.01 *M* increased glucose consumption in roots by 30 per cent in oxygen, but not in nitrogen. Glucose itself was accumulated in the roots (258).

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PHARMAKOLOGISCHES INSTITUT  
UNIVERSITY OF BERNE  
SWITZERLAND

## PHYSIOLOGICAL ASPECTS OF GENETICS<sup>1</sup>

BY M. R. IRWIN

*Department of Genetics, The University of Wisconsin,  
Madison, Wisconsin*

Practically every study utilizing genetic technics may well be said to have physiological implications of one kind or another. Therefore in this review it is impossible to include all the current papers of interest. A decision as to which papers to review had to be made quite arbitrarily, and this necessitated leaving out many which bear even on the topics discussed. Limitations of space did not allow the consideration of abstracts of papers presented before scientific societies.

Of general interest to biologists is the synthesis of studies on the biological basis of individuality by Loeb (1). Mention should also be made of a new textbook by Altenburg, revisions of well-known textbooks by Lush, and Snyder, respectively, and a reference book on chromosome numbers of various species of plants by Darlington & Janaki Ammal (2). An example of the growing interest in genetic problems by workers in other fields is a consideration of chromosome structure in a chapter by Greenstein (3).

*Chemical induction of mutations.*—Word-of-mouth reports from the British Isles during the past two years have hinted at the successful use there of mustard gas in producing mutations in *Drosophila*. A second preliminary report of this work by Auerback & Robson (4) is now available. These authors state that the rates of mutations produced by the treatment of *Drosophila* with mustard gas are comparable to those induced by x-rays. Both sex-linked lethals and visible mutations were produced. On the other hand, chromosomal rearrangements occurred at a lower rate relative to sex-linked lethals than with x-rays.

Another successful chemical induction of mutations is that of Hadorn & Niggli (5), who have obtained recessive mutants in the second chromosome of *Drosophila* after treatment of gonads *in vitro* with a 0.01 per cent solution of phenol. There was no effect from a comparable treatment with varying dilutions of colchicine. The gonads were dissected from the adults, and after treatment

<sup>1</sup> This review covers the period from September 1, 1945 to September 1, 1946.

transplanted back into host larvae. Some transplanted gonads became attached to the gonaducts, and the resulting offspring presumably came from the treated gonads. Strong (6) reports the appearance in mice of mutations involving coat color after successive generations had been subjected to a carcinogenic compound, methylcholanthrene. One change was from a recessive character to a dominant (brown to black), another from a dominant to a recessive (dark-eye to pink-eye), and changes in three types of spotting were observed. Other characteristics of a more general biological nature, such as larger litters than were typical of the ancestral stock, appeared in these stocks. The manner in which this carcinogenic agent appears to be able to affect the germinal tissue, presumably only after treatment over eight or more successive generations—as seemingly is required and which implies a cumulative effect—is indeed puzzling. These different findings are undoubtedly forerunners of an increased interest in the chemical induction of gene changes. Should they be corroborated in further experiments, current ideas of the stability of the germ plasm will require modification.

*Gene structure.*—An important paper by Stadler (7) describes observations on spontaneous mutations at a single locus in maize. What appear definitely to be a series of alleles at the R locus in maize affect the color of both seed and plant, as follows;

- R<sup>r</sup> Colored aleurone, colored plant
- R<sup>\*</sup> Colored aleurone, colorless plant
- r<sup>r</sup> Colorless aleurone, colored plant
- r<sup>\*</sup> Colorless aleurone, colorless plant

Spontaneous mutations of R<sup>r</sup> to R<sup>\*</sup> and r<sup>r</sup>, rarely to r<sup>\*</sup>, were noted in appreciable frequency. The point of greatest interest is that the frequency of the aleurone-color mutation (r<sup>r</sup>) is substantially reduced after the plant-color mutation (R<sup>\*</sup>) has taken place in R<sup>r</sup> plants (Cornell). It seems unlikely that this result would be obtained unless each mutation (R<sup>\*</sup> and r<sup>r</sup>) were parts of a single entity. Hence these findings definitely have implications on the concept of gene structure. However, since Stadler delayed discussion of these implications, it seems out of place for the reviewer to make any such attempt.

Based upon gene-enzyme systems in yeasts and somewhat re-

lated phenomena elsewhere [see previous reviews in this series by Wright (8) and by Danforth (9)], Lindegren (10) proposes a new theory of the structure of the gene. In this theory, the gene consists of two self-duplicating entities; namely, a chromogene which represents a place of attachment (or locus) in the chromosome for the active constituent, the cytogene. The cytogene, however, may multiply in the cytoplasm apart from the chromogene, and later become attached to the chromogene. There is no question but that the findings (previously reviewed) of Lindegren, Spiegelman and of Sonneborn in *Paramecium aurelia* need to be considered along with other more orthodox findings in arriving at an all-embracing theory of the gene. However, the acid-test of any theory is its ability to survive in the light of facts derived from experimentation.

Mampell (11) describes studies of a recessive gene in *Drosophila pseudoobscura* which causes a general increase in mutation rate, especially of Minutes and Minute mosaics. This "mutator" gene is in chromosome II, somewhere near the gene for cinnabar. There are certain peculiarities connected with the proposed action of this mutator gene. One is that when outcrossed, only males produce mosaics in their offspring. Another point is the assumption that the gene when homozygous produces a cytoplasmic component which induces the mutation, this component presumably being transmitted through the cytoplasm of the sperm. (The thought immediately comes to mind that passive immunity in animals is not known to be transmitted through the sperm.) Under this explanation some sort of self-duplication of this component in the cytoplasm is seemingly a requirement.

It is noted by Dunn & Caspari (12) that five mutant genes of the mouse, which produce similar abnormalities in early developmental processes, are closely linked. It appears probable that they do not represent "repeats" of a single locus, nor "overlapping deficiencies" as described in maize. Rather is it suggested that a segment of a chromosome carries several genes which when mutated affect early development processes—the end result in this case being expressed in a shortening or absence of the tail and a fusion of neighboring vertebrae. Usually genes affecting a character are scattered widely and seemingly at random over the chromosomes, as are those for eye color in *Drosophila* and for chlorophyll color

in maize. Unless this finding is exceptional, the principle involved is of considerable interest in the study of the genetic basis of complex physiological characters.

That genes with so-called major effects may also modify the expression of a character effected by another gene becomes more certain from the report of Blanc (13). Three genes with major effects in *Drosophila melanogaster*—pr for purple eye, ss for spineless, and b for body color inhibitor—also modify the expression of the vestigial character.

Stern *et al.* (14) report further studies to those previously reviewed (8, 9) dealing with the position effects of the normal and mutant alleles affecting "cubitus interruptus." The heterozygotes between the mutant gene ci and a normal allele  $\frac{+}{+}$  or  $\frac{+}{-}$  are generally normal in phenotypes. But if a rearrangement has taken place near the ci locus, the heterozygotes show varying degrees of vein interruptions. The distribution of the values of the average expression for twenty-six types of R(ci)/+ flies is significantly less extreme than that for nineteen types of R(+)/ci flies. This finding agrees reasonably well with expectation on the assumption that a new position of a gene involved in a position allele effects either a change in its functioning or a decrease in the amount of available substrate for its activity. These results are not in agreement with the explanation previously advanced by Ephrussi & Sutton of the position effect. These latter authors propose (15), following further experiments involving eye color, that their results support their hypothesis that position effect may cause a modification of stress near the affected loci. However, the possibility of the action of modifiers, or of competition between alleles, could not be excluded.

Ville (16) finds that alteration in the development of a part in *Drosophila* by the use of x-rays is possible as late as four to four and one-half days after development begins, much later than has generally been believed. This author (17) also tested all possible combinations of four homoeotic and of four "growth-rate" genes. He noted that one growth rate gene (dachs) has the same effect on the expression of the homoeotic mutants as if their larvae had been exposed to a low temperature during development; the other three (four-jointed, dachsous and combgap) have an opposite effect, i.e., an increase in the rate of development. Another example in *Drosophila melanogaster* of the interaction of genes (for Minute)

which delay development with various alleles of vestigial is given by Green (18). An effect of the Minute gene is to prolong the duration of the third larval instar, and this effect can be counterbalanced by a temperature of 30°C. Sensitivity to various temperatures in natural populations of *D. pseudoobscura* was noted by Dobzhansky (19), providing one explanation of a seasonal variation in the numbers of wild populations.

These current observations of genes which at least in part simulate the response to changes in temperature during development bring to mind Goldschmidt's proposals as to the two types of genic action; one type concerned with changing the relative rates of integrated developmental processes, the other with interfering with definite steps of chemical synthesis. Goldschmidt (20) adds further evidence in support of his thesis that certain environmental agents, as heat, can have the same effect as some mutant loci, and produce a nonhereditary modification, or phenocopy.

Pavan (21) states that there are two easily distinguishable types of heterochromatin in *D. nebulosa*. These are indistinguishable in the mitotic prophase and metaphase chromosomes, but one is reduced in the salivary gland chromosomes to only a few chromomeres, and the other forms the greater part of the chromocenter. Different types of heterochromatin have previously been reported in other species; the references are cited by Pavan.

The possibility that the longitudinal strings of condensation in salivary gland chromosomes may be artifacts is raised by Slizynski (22), who argues that they may be produced by an interaction in the staining process with alkaline urea or sodium thymonucleate. Ris & Crouse (23) propose that the giant chromosomes of dipterous larvae consist of a number of helically coiled chromonemata and that these are coiled so that they give the appearance of bands. This hypothesis has been criticized by Hinton (24), who suggests that the bands are isolated groups of genic material.

That phosphatase in salivary chromosomes appears to be distributed in bands more or less similar to the pattern of stained preparations is reported by Danielli & Catcheside (25). The apparent coincidence between sites of enzyme activity and genetic activity is suggestive of a process by which genes influence cellular activity. If this observation on the distribution of phosphatase is as well founded as the original work on the occurrence of phosphatase in preparations made from liver and later

work with other cells, it would seem that many results of fundamental importance may well be expected of it. A process denoting a priming of the chromosomes before their reproduction with desoxyribose nucleic acid from the nucleus is suggested by Darlington & La Cour (26), following observation of the effects of a new acetic-lacmoid smear technique followed by Feulgen staining of the large nuclei of liliaceous plants. These substances presumably represent a concentration of desoxyribonucleic acid in the nucleus; the proposed priming action on the chromosomes is a possible outcome.

*Genetic effects in bacteria.*—The so-called genetics of bacteria have aroused much interest in recent years. The absence of a sexual stage, at least one which is demonstrable, in the life of a bacterium is the chief limiting factor in such studies, to which must be added the uncertain state of our knowledge of the nucleus of bacteria. Two recent papers (27, 28) deal with this problem of nuclear structure. What have been called "dumbell bodies" (chromosomal stage?) are reasonably well established; their presence aligned in the long axis of the cells in certain bacteria is noted by Klieneberger-Nobel (27). The behavior of the chromatin of the spore mother cells of *Sphaerotilus notans* is suggestive of meiosis, there being four nuclear elements, three of which disintegrate and one presumably functions as the spore. On the other hand, Peshkoff (28) suggests, following studies of another species, that what have been called bacterial nuclei are simply solitary chromosomes, and comprise the genotype of the species.

Changes analogous to gene mutations have been observed in bacterial cultures following x-ray or ultraviolet radiation, and by what appears to be the selective action of various agents on a bacterial culture. Thus Tatum (29) irradiated with x-rays two mutant strains of *Escherichia coli*; one (strain 58) requiring biotin and the other (strain 679) threonine for growth. Of the seventeen mutants obtained, each of fourteen required only one of several growth factors for normal growth, in addition to the previous requirement of the particular strain. These substances included cystine, histidine, isoleucine, leucine, methionine, phenylalanine, proline, thiamin; one mutant strain required glutamic acid or proline, and three required unidentified substances present in yeast extract. The parallelism between these changes in the metabolic requirements of bacteria to those induced in *Neurospora crassa* is obvious.



Mutant strains of *E. coli* (B) which seemingly had lost their ability to synthesize tryptophane, and required various additional substances, were tested by Anderson (30) for resistance or sensitivity to different bacterial viruses (bacteriophages), with varying specificities displayed. Thus, five strains were resistant to virus T1; three to both T1 and T6; two to T1, T3 and T4; and seventeen to T1, T3, T4, and T7. In view of the studies of a decade ago by Levine & Frisch, who found that specificities in bacteria to the action of bacteriophages were usually if not always accompanied by antigenic differences in the bacteria, one other criterion of possible differences might well be added to the above reactions.

What appears to be the result of natural selection in *Staphylococcus aureus* towards various concentrations of penicillin is reported by Demerec (31). As the concentration of penicillin to which the bacteria were subjected was increased, different levels of resistance of the organism were noted. This is interpreted as being due to natural changes in the bacteria upon which the penicillin acts as a selecting agent—only those resistant can survive—rather than an effect induced in them by the penicillin.

Arguments that the changes in bacteria are the same, whether induced or occurring naturally, are advanced by Demerec (32), particularly in respect to their reaction to phage. At present, however, no distinction can be made in bacteria between changes involving a single gene from those resulting from chromosomal aberrations. According to Zamenhof (33) bacterial mutation occurs regularly in normal, healthy cells, which may be either young or old in respect to the time of division of the mother cells. Although x-rays and ultraviolet radiation usually increase the rate of the appearance of mutant forms in bacteria, there are some members of a bacterial culture which resist radiation. One such strain, itself presumably the result of mutation, is described by Witkin (34) in *E. coli*. The influence of the environmental factors which affect growth rates and viability is considered along with the mutation rate by Braun (35) in a study of dissociation in *Brucella abortus*. Considerable variation in mutation rates of this organism has been noted in different clones.

During recent years several workers other than those cited above have observed increased resistance to penicillin in various pathogenic bacteria, as a result of continual contact with the agent. One such paper (36) states that in addition to the increased resist-

ance of streptococci, staphylococci, and pneumococci to penicillin following contact with it, there was a decrease or even a complete loss of the virulence of these organisms towards mice. No such association was noted, however, in pneumococci by Schmidt & Sesler (37). More recently Miller & Bohnhoff (38) report that one strain of meningococci which would grow naturally in a medium containing 0.3 Oxford units of penicillin was gradually subjected to increasing concentrations of penicillin up to 18 Oxford units per cc. Ten organisms of the original culture were lethal to a mouse, but the virulence decreased with increased resistance to penicillin so that not even a billion organisms which would withstand 18 units of penicillin would kill a mouse. However, there were six other strains of meningococci which retained sufficient virulence beyond the 18-unit stage of resistance to penicillin to make them of significance as pathogens.

One of the most fundamental studies in biology of primary interest to geneticists is that of the transformation of pneumococcal types. Further studies on the transforming substance which can, in the presence of certain serous fluids or immune serums, induce an unencapsulated pneumococcus derived from Type I to produce the specific carbohydrate of Type III as well as more of the transforming substance itself are to be found in a series of papers by McCarty (39 to 42) and by McCarty & Avery (43). This transforming substance which was originally extracted from Type III pneumococcus appears to be desoxyribonucleic acid.

In attempts to determine whether the transforming principle is definitely the nucleic acid and not some other substance attached to it, the enzyme desoxyribonuclease was obtained from beef pancreas. In extremely minute amounts this enzyme would bring about a complete and irreversible inactivation of the transforming substance of Type III pneumococcus. The transforming substance could be inactivated by ascorbic acid, but under certain conditions the activity could be restored by the use of glutathione and other sulfhydryl compounds. All tests made in these experiments make it almost a certainty that the transforming agent is desoxyribonucleic acid.

Specific transforming substances have been isolated from pneumococci of Types II, IV. The biological activity of the substance appears to consist primarily if not entirely of desoxyribonucleic acid. No distinction between the desoxyribonucleates from these

different sources is possible on chemical grounds, but the transforming substance from each pneumococcal type is capable of changing an unencapsulated rough form only into the capsulated form from which the nucleic acid was derived, i.e., into Types II, III, IV, respectively. This would of course imply individual differences in chemical structure and molecular configuration among the respective nucleic acids extracted from these three pneumococcal types.

Also, a desoxyribonucleic acid can be extracted from unencapsulated R pneumococci which is similar in all respects to the Type III preparations, except that it is wholly inactive in the transforming system. Certain unencapsulated or rough (R) forms, seemingly not all, of pneumococci possess the potentiality of producing a carbohydrate specific to any one of several, possibly many, types of the organism and reproducing this type specific substance when supplied (a) with a particular transforming substance (desoxyribonucleic acid peculiar to the pneumococcal type) and (b) in the presence of certain serums or immune serums. Despite the ability of particular desoxyribonucleic acids (a) to induce in rough pneumococci predictable and heritable changes and (b) to reproduce in the transformed cells, in the light of our present knowledge, the transformation of types can be effected only when some constituent, as yet unknown, of serum is present. The nature of this property of the serum is indeed of importance in the further elucidation of these fundamental observations.

*Genetics of lower organisms*—The advances that have been made in this general field have been recently reviewed by Beadle (44), those in yeasts by Lindegren (45), as well as in former reviews of this series (8, 9). A short review of recent experiments with yeasts has also been given by Sansome (46). Various aspects of the genetics and specialization of parasitic fungi have been reviewed by Ingold (47), Reed (48) and by Johnson & Newton (49). Because of these various reviews, only brief mention will be made herein of recent papers bearing on this general topic.

Ryan & Lederberg (50) have found that a leucineless ( $l_1$ ) mutant in *Neurospora crassa* will spontaneously mutate to the wild type gene (L). The gene  $l_1$  is self-duplicating and may produce a "defective" enzyme in comparison with the active enzyme of the allele L. This situation is somewhat analogous to that of the rough form of pneumococcus which in the presence of immune serum will

occasionally revert to a smooth form of the same type. (As stated above the transforming substance will direct this change to a smooth form of another type.) The rough form of the pneumococcus—at least of type III—has a biologically inactive desoxyribonucleic acid which is chemically indistinguishable from the biologically active form. In *Neurospora*, the  $l_1$  gene is responsible for a defective enzyme, which becomes active when the gene changes. In the mutant *Neurospora* the defective enzyme may be activated; in certain rough pneumococci, the so-called "inactive" form of desoxyribonucleic acid may rarely become biologically active when in immune serum. At this point, our present knowledge for either organism of the process of the activation ends.

The effect of varying degrees of temperature on a mutant of *N. crassa* is given by Mitchell & Houlahan (51). This form requires less riboflavin for growth at low temperatures than above 28°C. Pierce & Loring (52) determined that a mutant strain which required for growth the addition of yeast extract to the medium gave the greatest unit weight if free adenine was added. Doermann (53) found that not only was it necessary to add lysine to the basal medium for satisfactory growth of a lysineless mutant, but that arginine was a specific inhibitor, and in the medium must be hydrolyzed to ornithine.

Horowitz (54) describes two mutants which are partially (strain 47904) or totally (strain 34486) unable to synthesize choline. The block to the synthesis of choline precedes the formation of monomethylaminoethanol in strain 34486; this compound cannot be synthesized but it can be utilized if provided. The other mutant (47904) can synthesize monomethylaminoethanol but cannot convert it into choline at the normal rate. In one mutant (34486) the block to choline synthesis precedes the production of monomethylaminoethanol, in the other (47904) it follows the formation of this substance. Other studies of the stimulation of the growth of mutant No. 34486 are given by Jukes & Dornbush (55).

How mutant strains of *Neurospora* may differ in blocking the synthesis of thiamin at different steps is shown by Tatum & Bell (56). One strain (18588) was blocked in the synthesis of thiazole, another (9185) in the coupling of thiazole and pyrimidine, the components of thiamin. According to expectation that other reactions in the biosynthesis of thiamin would occur normally, an accumulation of pyrimidine could be demonstrated in strain 18558,

and of both thiazole and pyrimidine in the other. Two other mutant strains (1090 and 17084) required for normal growth either thiamin or a mixture of thiazole and pyrimidine. Certain analogues of thiazole did not appear to be normal precursors of thiazole in *Neurospora*. These papers present additional examples to those previously reported that different reactions in the biosynthesis of a biologically important compound fail as the result of specific gene-mutations.

The factor of pathogenicity or virulence of lower forms of life towards either higher plants or animals has always presented many puzzling questions to biologists. An example of an association of virulence with a known composition of the organism is that of the carbohydrate structure of the different types of the pneumococcus. Such an association, however, is not universal. Preliminary findings by Keitt *et al.* (57), that certain genes for pathogenicity in *Venturia inaequalis* are linked with the sex reaction (58), indicate that genetic studies of this property are possible.

*Genic effects.*—A new gene ( $wx^*$ ) for waxy endosperm in maize, allelic to the wild type ( $Wx$ ) and waxy ( $wx$ ) genes, is reported by Brimhall *et al.* (59). This gene produces starch having 97.4 per cent amylose, the branched chain component. A higher content of nicotinic acid in the endosperm of twenty-four strains of maize with sugary endosperm than in five strains of flint or dent varieties is described by Mather & Barton-Wright (60). This effect was primarily but not entirely associated with the starchy and sugary genes ( $Su-su$ ). No definite difference in content of nicotinic acid was observed between yellow and white endosperm. This effect then appears to be quite independent of the content of vitamin A, which was reported many years ago (61) to be correlated with the number of genes for yellow pigment.

Doubling the chromosome number of maize was accompanied by an increase in the amount of nitrogen present in the grain and stalks, according to Ellis *et al.* (62). Other materials, crude fiber and ether extractable components of the grain, and ash, lignin and ether extractable material of the stalks, were present in essentially the same amounts in the diploid and tetraploid forms. Similar results were obtained by Chen & Tang (63) of the nitrogen content of the seeds of colchicine-induced autotetraploid barley. These workers found more lipid substance and ash in the tetraploids than in the diploids, and a lower rate of oxygen consumption and of

carbon dioxide production was noted in germinating 4n than in 2n barley seeds. There were also noted differences between the 2n and 4n seeds in enzymatic activity (64), the 2n seeds being higher than 4n in the activity of certain enzymes, lower in others.

The action of the gene (h) for hemophilia in man is still a puzzle as to just what stage in the clotting of blood is interrupted. New hope for a normal life is held out to those afflicted with this recessive genotype, as a result of work recently reported by Minot *et al.* (65) and Lewis *et al.* (66). Hemophilic blood appears to be deficient in a factor which is closely associated in chemical fractionation with prothrombin and fibrinogen. This factor may be partially separated from both these proteins. Injections into hemophiliacs of a fraction of plasma with 60 to 70 per cent of fibrinogen and smaller amounts of other globulins were generally successful in reducing the coagulation time of the blood towards a normal value. Thus the effect of a gene in interrupting normal physiological development may be counteracted by an extraneous treatment.

Another example of how to modify the usual expression of a genotype for plumage pattern in poultry is given by Juhn (67). The F<sub>1</sub> males of the cross between Barred Rock and Brown Leghorn have some feathers with barred apices and Leghorn-like bases among the predominantly barred plumage. A depression in the metabolic level of these birds is produced by placing them on a continuous thiouracil diet. As a result, barring appears in the basal section of Leghorn-like feathers.

Whiting (68) subjected unmated females of *Habrobracon* to x-rays and then mated them to untreated males with recessive characters. There were a few males among the progeny which carried the recessive phenotypes of the male parents. In addition, breeding tests showed that their genotypes were paternal rather than maternal in origin. This finding is quite in contrast with the usual expectation of the genotype of haploid male offspring in Hymenoptera.

*Cytoplasmic influence.*—Susceptibility of *Drosophila* to carbon dioxide behaves as if it were hereditary, but in crosses it follows non-Mendelian rules. A paper by L'Heritier & de Scoeux (69) reports success in transmitting the susceptibility to part of the wild-type offspring, following the grafting of ovaries from wild-type resistants to mutant (ebony) susceptible hosts and then mating to ebony males. The three females with functional implanted ovaries resulting were susceptible to carbon dioxide. These results differed

from those of Kalmus & Mitchison (70) but are not necessarily contradictory because of the small number of successful grafts obtained by the respective workers.

Owen (71) suggests that although some types of male sterility in sugar beets are produced by genes, at least one type is the result of the interaction of genic and cytoplasmic factors. This cytoplasmic factor for male-sterility was generally stable, although some instances of instability were noted.

The importance of the endosperm in a developing seed in providing nutriment for the embryo is emphasized by Cooper & Brink (72). The failure of seed to develop from crosses between a diploid and its autotetraploid in *Lycopersicon pimpinellifolium* is comparable to that occurring in crosses between two species. (This general subject will be treated more fully in a forthcoming review by these authors.)

*Heterosis*.—The reader is referred to two reviews on heterosis for general considerations of the subject; one in 1944 by Whaley (73), the other by Mather (74). The first of a series of papers by Gowen and co-workers (75), using the criterion of egg production in *Drosophila* as the character to be measured, sets the stage for succeeding papers. (One of these has appeared as this review goes to press.)

Recent observations by Jones (76) cast new light upon the question as to how heterosis can become manifest. Six recessive mutations, presumably involving single genes which would be classed as degenerative changes, have appeared in five inbred lines of maize. The striking point is that the progenies from matings between each mutant line and its parent line showed heterosis. Both yield of grain and height of stalk were greater than in either immediate parent. In other words, hybrid vigor in each case seemingly resulted from the interaction of two alleles. These findings, coupled with those of Stadler (7), provide experimental backing for the theoretical but rather accepted concept of hybrid vigor as dependent upon the interaction of alleles.

*Species and species crosses*.—Sanz (77) reports studies of the growth of pollen tubes on the stigma and styles of *Datura stramonium*, using pollen from various other genera. If the styles were tetraploid, the speed of the growth of the pollen tube was slightly increased; if the length of the style was decreased, the pollen tubes approached closer to the ovary, but in no case did they enter it.

Intercrosses of races of *Rana pipiens* from various geographical



locations in the United States give offspring with varying degrees of abnormality, according to Moore (78). Every race, however, can be crossed successfully with another species, *R. palustris*. Sturtevant (79) reports an autosomal gene in *D. neorepleta* which, in single dose in hybrid females obtained from matings with *D. repleta* affects the eggs of these females so that the eggs have a potentiality for maleness, resulting in intersexes.

Preferential mating between males and females of *D. pseudoobscura* and *D. persimilis* and their hybrids has been tested by Mayr (80, 81). In general the males of either species prefer to mate with the females of the same species (8); however, the males of *D. persimilis* mate more often with the hybrid females than with those of their own species; the reverse situation is true of the males of *D. pseudoobscura*.

Patterson (82) describes a physiological response to mating which may well be a barrier to species crosses in many species of *Drosophila* and possibly in other forms as well. Shortly after mating, the vagina begins to enlarge until it reaches a size several times that of the organ in virgin females. In intraspecific matings, the vagina usually returns to its normal size in a few hours. In interspecific matings it may maintain the increased size for several days, with partial to complete loss of ability to perform its normal function. The term "insemination reaction" is proposed for this phenomenon. It appears that this reaction occurs as a result of the semen—not the sperm—coming in contact with the mucous membrane of the vagina. The response then simulates that of sensitized tissues to a foreign protein. The sensitization in this instance must be an inherited characteristic since this reaction occurs in the females at their first copulation. This observation has many ramifications which can be tested experimentally.

*Genes and antigens.*—The importance of the Rh (rhesus) and Hr antigens in human blood cells is well established. Although workers in the field did not at first agree on the genetic relationship of these two antigens, it is now reasonably clear that they are contrasting characters, as has been held by Levine (83). The various subtypes of the Rh antigen, and the two or more of Hr, are currently the subject of debate as to whether these are produced by (a minimum of six) allelic genes as is proposed by Wiener, or by (three pairs of) linked genes, a concept upheld by Fisher, Race and colleagues. Recent papers by Wiener *et al.* (84) and by Fisher &

Race (85) present evidence in support of their respective views. (In the interest of clarity, the reviewer wishes that an agreement on the nomenclature of the various subtypes could be reached.)

These various subtypes are recognizable to date only by specificities in the serums of women, usually only in serum from those who have delivered infants afflicted with erythroblastosis fetalis. By virtue of a different specificity of reaction with various cells of known phenotype, a new subtype of Rh is reported by Stratton (86). Also, an antigen seemingly independent serologically of any of the previously known complexes of antigens in human cells—AB, MN, P and Rh complexes—is described by Mourant (87). Many more will undoubtedly be found, one limiting factor being the antibodies to detect them.

One of the puzzling questions in this field is why a relatively small percentage of women lacking Rh become sensitized to it. On theoretical grounds alone, Wiener (88) postulates that the ability to become sensitized is itself an heritable character, dependent upon a dominant gene (K). The homozygote (KK) represents those rarely found individuals who become sensitized during the first pregnancy.

Contrary to early opinions that the Rh antigens were found only in the blood cells, they have been noted in tissues other than the blood cells and recently have been found in the amniotic fluid (89). Furthermore, there appear to be both secretors and non-secretors (89) of Rh as there are of the A and B substances, but a different enzyme system is seemingly involved for each genetic system of antigens. This brings up the question of whether the substances from secretors or nonsecretors would be more likely to immunize the mother. In three cases of Rh-negative mothers with erythroblastotic infants (89), the three Rh-positive infants were nonsecretors. On the other hand, Smith (90) reported twenty-four of forty cases in which the child possessed an antigen (either A or B) not found in the mother, and in which there had been a rising antibody titer corresponding to the antigen of the blood of the child. The mothers who failed to show a rising titer had non-secretor children; the secretor children produced iso-immunization of the mothers. Whether this parallels immunization with the Rh antigen can only be decided after further tests. It might be assumed that iso-immunization of the mother would be more readily accomplished by substances outside the blood cells, rather than

disintegration products of the cells. These same substances, however, might be expected more readily to take up the immune bodies from the mother's serum, thereby preventing injury to the cells of the fetus. Several cases of erythroblastosis fetalis have been reported (91, 92) in which the Rh antigen presumably is not a factor. Those reported by Poloyes & Ohlbaum (91) suggest that the A, and possibly the B antigen was involved.

The relationship of antigenic characters in different species is of general interest. Thus antigen A of man is found seemingly *in toto* in some chimpanzees, as is a single antigen related to, but not identical with, the contrasting antigens M and N. The Hr substance—not the Rh—is present in chimpanzees, and appears to be indistinguishable from this component of human cells (93, 94).

The blood cells of hybrids between Senegal (*Streptopelia senegalensis*) and Ring dove (*S. risoria*) showed individual differences in antigenic content (95). These differences could be explained by a segregation of antigens related to the d-1, d-2 and d-4 of Pearlneck (*S. chinensis*). Although the antigens peculiar to Senegal were not then identified as units, their general distribution in the progeny of the species hybrids mated to Ring dove appeared to be as expected on a chance basis (96). This suggests independent assortment in the hybrids of the chromosomes of Senegal carrying the causative genes, and more or less normal pairing of these chromosomes with potential partners of Ring dove.

An interesting finding in cattle has been made by Owen (97), in that the combination of cellular antigens in the majority of over eighty twin pairs was identical for each pair. In man, fraternal twins are not more alike than are sibs, whereas identical twins are always of the same type. The well-known vascular anastomosis between bovine twins *in utero* could readily explain the interchange of blood cells. Their persistence to adult age in either twin, however, requires the proposal that embryonal cells ancestral to the erythrocytes of the adult animal may become established in either twin and produce cells thereafter. The existence of two kinds of blood cells in such twins can be demonstrated in the laboratory, adding further evidence of the reasonableness of the above proposal.

It is known that the serum proteins of most if not all animal species are different, and that genes have effects on these proteins. Of recent years the electrophoretic patterns of the serums of many species have been studied by means of the Tiselius apparatus, pri-

marily with the objective in mind of separating a serum into its different proteins. In general, when examined under comparable conditions, the number and relative quantity of the components of the serums varied significantly with the species. The results of this approach are summarized and newer findings are given by Moore (98). This author observed differences in the serums of two strains of rats, and between males and females in chickens. Many years ago it was proposed that the serums (proteins) of roosters and laying hens could be differentiated serologically. On the other hand, attempts (99) to distinguish the serums of pigeon (*Columba livia*) and Ring dove by the pattern of their electrophoretic mobilities were unsuccessful, although a differentiation was possible serologically. More electrophoretic studies should be made of the serum proteins from animal species which hybridize.

Only passing reference will be made to a study by Moore *et al.* (100) of the electrophoretic pattern of the plasma of developing pig and chick embryos. Rapid changes could be detected. Fox (101) noted that each of four rabbits differed in the axial wave length of the  $\alpha$ -band of oxyhemoglobin. No such differences were observable, however, in the blood of humans from widely differing origins.

Evidence of an interchange of cytoplasm in *Paramecium* has depended largely upon deductions based on the time in conjugation, and the observance of cytoplasmic streaming. Recently, Harrison & Fowler (102) found alterations in the antigenic composition of conjugants in *P. bursaria* which they interpret as being indicative of an extensive interchange of cytoplasm during conjugation.

*Genetics and pathology.*—It may well be said that the establishment of strains of animals or plants which are resistant or susceptible to particular infections, or neoplasms, is but the first step in the search for knowledge of the interactions between host and pathogen. Nutritional factors present in whole wheat appeared to promote resistance against *Salmonella enteritidis* in one strain of mice, but not in three inbred strains (103). Scholes & Hutt (104) believe that chicks with a higher body temperature are more resistant to *S. pullorum* than those with a lower temperature, but this is held by Severens *et al.* (105) to have only an indirect effect on natural resistance. These latter workers propose an association of hereditary resistance with the number of lymphocytes in the blood. Irradiation with x-rays lowers the number of lymphocytes

and also lowers the resistance of the treated birds. Gowen and co-workers (106, 107) find that a high number of leucocytes per cc. of blood—not any single type of leucocyte—characterizes two strains of mice resistant to *S. antrycke*; two other susceptible strains have a lower number of leucocytes, and two strains intermediate in resistance have an intermediate number of leucocytes. A lowering of resistance is produced by irradiation with x-rays; the susceptible strains become highly susceptible. Oakberg (108) reports that the splenic tissue from these intermediate and resistant strains had few lesions, that from the susceptible strains showed extensive lesions. On the other hand, liver tissue from the resistant strains had more lesions than did that from the strains of intermediate or low resistance. The fixed macrophages may also be an important factor in overcoming the inroads of the pathogen. Somewhat as the disputes between the proponents of the humoral and cellular theories of resistance, respectively, were reconciled by experiments showing that each theory, or both, held in particular cases, so may it be assumed that there are different mechanisms working in various species to make for natural resistance, at least to different organisms.

An interesting and promising attack on the question of the mode of expression of natural resistance to a microorganism has been initiated by Huff & Coulston (109). These workers follow the varying degrees of resistance to the malaria-producing organism (*Plasmodium*) which are displayed by different species of birds. The sporozoite and erythrocytic stages can be traced in the host tissues and the point at which their development is retarded in their attack on the host can be determined, and correlated with the degree of resistance.

Genetically resistant and susceptible strains of plants to various pathogens are commonplace in plants as compared to their more or less rare occurrence by selection in animals. Unfortunately an understanding of what lies behind the genetic resistance in plants is precise in only a few isolated instances. For example, pigmented onions are resistant to the organism causing smudge (*Colletotrichum circinans*) by virtue largely of their content of catechol and protocatechuic acid in the (dry) colored scales. However, this resistance disappears in white bulbs with a dominant inhibitor (I) for color (110). Cream colored bulbs (Ii) are approximately intermediate in resistance between full white and colored varieties.

Variations in the results of symbiosis between legumes and nodule bacteria have heretofore been recognized as caused primarily by variation in the bacteria. That symbiosis may also vary because of genetic differences in the host is reported by Nutman (111). The formation of root-nodules, then, is another example of a host-parasite relationship.

Because of the appearance of a comprehensive review (112) on mammary tumors in mice, only brief mention will be made of the so-called "milk influence." Bittner & Huseby (113) consider that mammary cancer in mice is a resultant of (a) the milk agent, (b) the inherited susceptibility of the individual or race, and (c) hormonal stimulation or influence. Further confirmation of the importance of the innate influences is provided by the experiments of Miller & Pybus (114) and of Heston and colleagues (115).

The genetic factors for length of life appeared to be more or less independent from those making for spontaneous tumors in hybrids in rats (116) and for leukemia in mice (117). An inverse relationship of the advancing age of the mothers and the incidence of leukemia in the offspring was also noted by MacDowell *et al.* (117).

The use of immunological technics in the study of tumors has given results of interest in some experiments and might well be employed more widely. For example, Nettleship (118) found that the rate of growth of a transplanted lymphosarcoma (Murphy) in the rat could be retarded and the mortality reduced by the use of immune serums produced in the rabbit to various fractions of the lymphosarcoma material. The specificity of the resistance induced in C<sub>3</sub>H mice by the inoculation of material from a sarcoma was tested by Gross (119), who found that protection against one type of tumor cell did not mean protection against another originating in the same inbred strain of mice. This finding conforms in principle to those of earlier experiments in which different specificities of transplantable tumors have been observed by means of biological assays.

*Genes and morphogenesis.*—A method which will allow a precise analysis of the respective influences of the maternal environment and genotype of the embryos is reported by Russell & Hurst (120). This consists in the transplantation of fertilized ova to females. It should be applicable in various other problems of genetics.

A histological study of the pigment of the coat-color mutants (thirty-six genotypes) in the mouse by Russell (121) has made use

of the knowledge that the processes which have gone on during the life of a hair bulb are laid down within the hair itself from the tip to the base. Variation was found among the granules: (a) in number of granules per medullary cell; (b) in number of cortical granules per unit volume; (c), (d), and (e) in the distribution, size and shape of the granules within medullary cells; (f) in the clumping of granules; and (g) in color of the pigment granules. All these variations play a part in making the final coat-color of the different genotypes.

The loss of hair in "hairless" and "Rhino" mice accompanies a widening of the hair canal and consequent lack of the support supplied to the hair normally by the tight-fitting follicle neck (122, 123). A transplant of rhino skin to a normal host remains more or less intermediate in growth of hair and in thickness and wrinkling of skin, as if this implanted tissue were able to utilize some tissue from the neighboring epidermal cells which it itself could not produce.

The extragenic factors which may produce rumpleness in chickens have been reviewed (9). A further report on the use of insulin in producing rumpleness has appeared (124), as well as that of a second type of rumpleness recessive in nature which has several distinguishable characteristics from the dominant form (125).

A confirmation of the beliefs of various workers that the stimuli which lead a fly to its food (chemoreceptivity) may be largely localized in the antennae of some species is supplied for *D. melanogaster* by Begg & Hogben (126). The peg-like organs or cones, or both, on the surface of the distal joint of the antennae appear to be chemoreceptors.

An effect on coagulation of blood in man different from the more-or-less unknown effect of hemophilia (h) is that of a total absence of fibrinogen in the blood, hence complete incoagulability of the blood of those affected (127). There are many other reports of genetically produced abnormalities in man and other animals, but limitations of space do not permit reference to them.

In conclusion, it may be stated that hopeful progress has already been made in an understanding of the gene itself and of the physiological effects of genes. The reviewer, however, is reminded of a remark made recently by a well-known organic chemist, that the present understanding of chemical reactions in relation to



those yet to be explained might well be compared to a map of the United States which would have been drawn by a geographer in the early years of the seventeenth century. If this is true of the present knowledge of the known chemical reactions in relation to those unknown, how much more true is it of the present knowledge of biological reactions.

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DEPARTMENT OF GENETICS  
UNIVERSITY OF WISCONSIN  
MADISON, WISCONSIN

## DEFENSE MECHANISMS<sup>1</sup>

WILLIAM C. BOYD<sup>2</sup>

*Department of Biochemistry, Boston University School of Medicine,  
Boston, Massachusetts*

AND

SAUL MALKIEL<sup>3</sup>

*The Rockefeller Institute for Medical Research, Princeton, N. J.<sup>4</sup>*

When the natural barriers of the body fail to prevent a pathogenic microorganism from gaining entrance, an infection results, and defense mechanisms begin to operate. Everyone is familiar (30) with the typical cause of a human infection such as that following a cut in the skin, or a boil. Inflammation (124 to 127) sets in, leukocytes are mobilized, and antibodies may be formed.

*Cellular mechanisms.*—In an extensive series of articles Menkin (128 to 142) has elaborated on his previous studies on cellular inflammation. From inflammatory exudate he has isolated fractions which he has termed pyrexin, necrosin, and leukocytosis-promoting factor. Necrosin was found to be associated with the euglobulin fraction and presumably was liberated from cells which had been initially injured by an irritant. This fraction was markedly antigenic and produced a serum with a high antibody titer. In experimental animals necrosin produced marked inflammation, redness, edema, superficial necrosis, leukocytic response, lymphatic occlusion by fibrin, and a leukopenia which was often followed after several hours by a rise in the number of circulating leukocytes. Pyrexin, a pyrogenic factor, was apparently formed by the enzymatic action of necrosin. Leukotaxine, the leukocyte-promoting factor, was found to be associated with the pseudoglobulin fraction of exudates and was reported nonantigenic. Leukotaxine was found to be related to the discharge of immature leukocytes from the bone marrow and caused a marked hyperplasia of the granulocytic and megakaryocytic elements of the bone marrow. It was found to be present only in the serum of an animal having a concomitant acute inflammation.

<sup>1</sup> This review covers the period from 1942 to 1945.

<sup>2</sup> John Simon Guggenheim Memorial Fellow, 1935-36.

<sup>3</sup> Senior Fellow, American Cancer Society.

<sup>4</sup> Visiting Investigator.

*Immunity vs. hypersensitivity.*—The changes which occur in an organism in response to an infection usually enable it to throw off the infection, and are thus beneficial. Noninfectious dead microorganisms may also provoke alteration and apparently harmless substances may sometimes cause alterations in the tissues or circulation. Burnet (30) has supposed that the basic mechanism consists in the recognition by the tissues of the organism, of the difference between "self" and "not-self," followed by measures designed to eliminate the "not-self." The defense which an organism puts up against infection does therefore seem somewhat analogous to the processes of digestion, as Metchnikoff thought long ago. This analogy is not weakened by the important role which we now know is played by antibodies, which themselves seem to have no digestive power. "Defense" measures, in the case of harmless substances such as ragweed pollen, may set up a mode of reaction which is actually deleterious to the organism. We can only suppose that, on the whole, the benefits outweigh the harm, or otherwise the device of immunity-sensitization would not have survived in the long course of evolution.

No discussion of chemotherapeutic methods is attempted here. The discovery of strains of microorganisms which have become resistant to sulfa drugs (194, 212), penicillin (13), and streptomycin (29, 144, 215), just as trypanosomes have in the past become "drug-fast," might suggest that chemotherapy is unlikely completely to replace immunological methods in the near future.

### ANTIBODIES

*Antibody specificity.*—The importance of antibodies in resistance to disease has become clearer in the light of recent investigations, although some puzzles remain to be explained. It would be impossible to list all the evidence. We may mention, as outstanding new contributions, the demonstration of a circulating antibody for mumps virus and proof that resistance correlates well with the presence of the antibody (63). Other antibodies (105) have found practical application. We may call attention to such facts as the observation that the serum of the great majority of adults seems to contain antibodies capable of neutralizing considerable amounts of influenza A virus (32).

The specificity of antibodies is fully discussed in the masterly second edition of Landsteiner's book (115). The reader will find

the chemical bases of specificity thoroughly covered there, with a wealth of detail impossible in a review such as the present. Most of the work, in any case, was published before the period of time we desire to cover.

The specificity of antibodies often becomes less as immunization is continued, as shown by the increase in strength and extent of cross reactions (115). To some extent this may be due to increased antibody concentration in the sera from later bleedings, but some observations suggest that it is partly caused by the production of antibodies of greater combining capacity (115). Qualitative changes in combining power may also be observed between early and late antibodies, for it has been shown that in at least one case the later antibodies could combine with a (related) antigenic determinant with which the antibodies at first produced no reaction (99).

The importance of acidic groups in antigenic structure has been stressed by Landsteiner (115). Haurowitz (83) has shown that strongly basic groups are probably just as effective as acid groups in directing specificity.

Different antibodies have been found to vary in resistance to high pressures (20), heat, acid or alkali (112, 115). Aggregation of the protein molecules in the serum also takes place (9). Other differences have been found; agglutinins for the flagellar antigens of the typhoid bacillus are more resistant to heat than are the antibodies to the somatic O antigens. These same agglutinins can be largely separated by fractionation of serum with alcohol under suitable conditions (39).

The sedimentation constants of various antibodies have been determined. In man and the rabbit, the sedimentation constant (about 7 Svedbergs) and diffusion constant indicate an antibody molecule of about the same size as normal serum globulin, i.e., with a molecular weight of about 160,000. In some animals such as the horse, cow, and pig, a much larger molecule, with a molecular weight of about 1,000,000, may be found. In some species small amounts of an X globulin are found, with a less well-defined sedimentation constant, in the neighborhood of that of serum albumin, but varying greatly with the density of the solvent (162). The exact relation of this to antibody-antigen reactions is still uncertain. Davis *et al.* (42) found that Wassermann antibody (syphilitic reagin) dissociated from the antibody-antigen precipi-



tate showed two components, with sedimentation constants of 7 and 19 respectively. Other workers have reported a high molecular weight for this antibody (cf. 115).

The electrophoretic patterns of antibody preparations are now generally used as an index of homogeneity (though not necessarily of purity). Rabbit antibody seems generally to have an electrophoretic mobility corresponding to that of the  $\gamma$  globulins. Antibodies and normal  $\gamma$  globulins seem to have about the same chemical composition.

Seibert and Nelson (186) found that an antibody to tuberculin protein in rabbits had about the electrophoretic rate of an  $\alpha$  globulin. Davis (42) found that the Wassermann antibody (syphilitic reagin) dissociated from precipitates formed with the antigen has a mobility between that of the  $\beta$  and  $\gamma$  globulins.

*Purification of antibodies.*—At low temperatures (about 0° C.) antibodies can be fractionated by alcohol (38, 66, 67). The temperature, pH, protein, and alcohol concentrations, and the ionic strength (dependent upon the salt concentration) can all be varied. Plasma was separated (36) into four main fractions I (containing most of the fibrinogen), II+III (containing most of the globulin), IV (mostly globulins), and V (largely albumin). Antibodies present in the plasma were found in fraction II+III. By subfractionation of II+III, relatively pure globulin could be obtained.

Antivirus antibodies capable of protection against measles and infectious hepatitis have been found in the  $\gamma$  globulins separated from plasma (156, 198, 199).

Enzyme treatment has also been used (153, 157). Rothen (177) found that antitoxin purified by means of enzymes had a sedimentation constant of 5.5, while that which had not been so treated had a sedimentation constant of 6.9, probably indicating that the enzyme-treated material has a somewhat lower molecular weight. The work of Peterman (166) is interesting in this connection.

*Antibody formation.*—Most of the theories of antibody formation proposed in the last twenty years suppose that antibody is serum globulin, built up in the presence of, or modified by contact with, the antigen (2, 27, 151, 158). However, Burnet (31) does not think there is any adequate reason for believing that simple juxtaposition of the growing antibody molecule to the antigen would result in a complementary pattern. Instead, he thinks that it is more likely that certain proteinases (or other enzymes also?)

in the antibody-forming cells are lastingly modified as they are engaged in destroying the antigenic particles. Then these "trained" enzymes synthesize the antibodies. This hypothesis has the merit of a certain plausibility; data to prove it are still lacking, as in the case of the older hypotheses. Cannon (33) has called attention to the probable importance of an adequate supply of protein in the diet for adequate antibody production.

It has long been believed (35, 73, 84, 103, 104, 106, 178, 179, 193; cf. 203) that antibodies are formed in the reticuloendothelial system. More recently, considerable evidence has accumulated (47, 48, 49, 59, 60, 61, 82, 110) that lymphocytes form antibodies. Ehrlich & Harris (60) believe that the granulocytes may play an essential role. On the other hand, the importance of the role of the reticuloendothelial system is not diminished by these new findings according to Kabat (107); however, Ehrlich *et al.* (61) have failed to find evidence that the macrophages form agglutinins against the microorganisms used in their experiments (dysentery and typhoid bacilli). According to Dougherty *et al.* (48), the "anamnestic" response is dependent upon the release of antibody from lymphocytes as a result of pituitary-adrenal cortical stimulation.

For years there has been an almost mystical belief that contact with, or incorporation of part of, the antigen, was necessary for, and would explain the specificity of, the corresponding antibody. The idea of incorporation has been disproved (cf. 18), but the same habit of thought continues to crop up in different guises, in spite of the more general belief, based largely on Landsteiner's work, that the affinity between antibody and antigen is purely chemical in nature (115). Thus Pauling *et al.* (159) have claimed the artificial production of antibody *in vitro* by "undenaturing" serum proteins while they were in contact with antigens. It does not seem that any outstanding immunologist at the present time believes that any real antibody has ever been made artificially (107). Even less plausible are the claims of Rosenow & Johnson to have produced antibodies by heat or pressure treatment of bacteria (176). An impartial survey of the field does not indicate that the belief that each antigen will be found to generate *in vitro*, or to be associated with, the appropriate antibody, is any better founded than the old dictum "*similia similibus curantur*."

Certain workers (209) believe that certain kinds of antibody, such as "inhibiting," "incomplete," or "blocking" (173) antibodies

are univalent, and other antibodies are divalent or multivalent. The "valence" (number of specific combining groups per molecule) of antibody is still somewhat uncertain.

Pauling and co-workers (160) have calculated the valence of antibodies from analytical studies on precipitates made with various haptens. Objections, however, can be raised to their experimental technic. It is also likely that many, and perhaps most, of the haptens used by Pauling are not molecularly dispersed in solution (22), or, in other words, are aggregated. If haptens are aggregated, the valence of each particle of hapten is correspondingly greater than computed from simple chemical formula, and, little reliance can be placed on Pauling's calculations of antibody valence. Some observations suggest that antibody does usually have two (or possibly more) combining groups (1).

*Quantitative estimation of antibodies.*—Heidelberger & Treffers (92) have estimated the total hemolysin in lytic sera by determining the nitrogen added to sheep stromata suspensions. Henriksen & Heidelberger (95) have applied a quantitative agglutination procedure to the determination of the amount of antibody in antisera to hemolytic streptococci. Heidelberger & MacPherson (90) have described a method of estimating very small amounts (as little as 10  $\mu$ g.) directly as protein.

Antihormones are discussed in a paper by Thompson (201).

## ANTIGENS

The use of adjuvants to obtain enhanced antibody response in immunization is becoming more popular. In particular, the procedure introduced by Freund & McDermott (70) of incorporating the antigen in a lanolin-like substance (aquaphor or falba), which is then emulsified with a suspension of tubercle bacilli or other acid fast organisms, is fairly widely used (71, 107).

Introduction of too much hapten into conjugated antigen may diminish the antigenicity of the conjugate (26, 115).

*Antigenicity.*—The conditions of antigenicity have been discussed by Boyd & Malkiel (23). Much uncertainty regarding this question still remains. Purified specific capsular polysaccharides of the pneumococcus are devoid of antigenicity for rabbits and horses, but they can, under proper conditions, call forth the production of specific antibodies in a man or a mouse (52). The reason for this difference is not understood. It has been proposed that the non-

antigenicity of gelatin is due to the relatively rapid elimination of this protein from the circulation (85). This same characteristic seems to be the cause of the relative inefficiency of gelatin as a blood substitute.

One animal toxin, crotoxin from the rattlesnake *Crotalus terrificus*, has been crystallized (191). It is a protein which seemed to be homogeneous and to possess a molecular weight of about 30,000 (115). Both the toxicity and the hemolytic effect seemed to depend on the lecithinase activity of the molecule. Cobra venom (115) has also been purified. Venoms seem to have considerable sulfur (4 to 5 per cent) and from 5 to 6 per cent of zinc.

Lamanna *et al.* (114) have recently purified and crystallized botulinus type A toxin. It seems to be a protein with a molecular weight between 1,000,000 and 2,000,000. The MLD (for mice) was found to be about  $8 \times 10^{-9}$  mg., in terms of nitrogen. Pillemer *et al.* (170) have isolated and crystallized tetanus toxin. It also behaved as a protein, and the MLD for mice was about  $1$  or  $2 \times 10^{-8}$  mg. of nitrogen.

Furth & Kabat (72) found materials of high molecular weight in all normal and neoplastic tissues. The Forssman and Wassermann activity seemed to be carried by these substances.

Avery *et al.* have shown (8) rather conclusively that the substance, which brings about the transformation of rough (uncapsulated) pneumococci into smooth (encapsulated) type 3 pneumococci, is a highly polymerized, viscous form of desoxyribonucleic acid. Similar substances would induce the transformation to other types (119). This discovery, which does much to clear up the mystery hitherto surrounding the mechanism of the transformation, has far-reaching implications for all future thinking about development in general.

A number of methods of releasing the carbohydrate from various microorganisms in a fully antigenic form have been devised (43, 52). These methods include extraction with trichloroacetic acid (14), phenol (146), diethyleneglycol (148), etc. Freeman (69) obtained the antigenic complex of *S. typhimurium* by alcohol and ammonium sulphate fractionation. The product consisted of a mixture of at least two substances having sedimentation constants of approximately 57 and 60 respectively. Hydrolysis by acetic acid gave (a) a specific polysaccharide (69 per cent), (b) an insoluble conjugated protein (16 per cent), (c) a mixed lipid fraction (3 to 4

per cent), and (d) a nonantigenic, alcohol-soluble carbohydrate (about 8 per cent).

Peluffo (163) has reported results which suggested that the stability of the Vi antigen to other destructive agents is greater than previously supposed, and he believes that, when dehydrated, it resists heat fairly well.

The fact that anti-Vi antibody agglutinates cells containing the Vi antigen and protects animals against experimental infection with Vi organisms indicates, according to Dubos (52), that this antigen is present at the cell surface. The O antigen is considered to be nearly equally superficial, but the Vi antigen is evidently more so, as its presence can prevent agglutination of O cells by anti-O serum (3). Almon believes that organisms containing the Vi antigen multiply faster than do those lacking it because of the protective action the Vi antigen exerts against the phagocytes.

A good many studies have been made on the virus of influenza, especially during World War II. The results were at first not too consistent, for Chambers *et al.* (36) calculated that the particles of influenza A are about 110 Å in diameter, whereas Sharp *et al.* (189) estimated about 1150 Å. This discrepancy has recently been resolved, and there is now general agreement that the size of the influenza virus is about 1000 Å (195).

### BLOOD GROUPS

A blood group O substance has been obtained (150), and precipitins acting on the O substance have been reported (25). Morgan (147) showed that artificial complexes of (hog) A substance with the conjugated protein from Shiga bacillus would produce, in rabbits, powerful and specific anti-A sera.

Purified A and B substances, prepared from hog and horse stomach respectively, have been found useful in lowering the anti-A (and anti-B) titer of group O "universal donor" blood before transfusion (214). Studies of purified A and B substances have been reported by Kabat *et al.* (108).

Probably the best method of routinely obtaining strong and specific grouping reagents is the method of Witebsky, Klendshoj & McNeil (213) of injecting purified A and B substances (from hog and horse respectively) into human volunteers. This procedure seems entirely safe, and gives good sera in about half of the volunteers. A carbohydrate which is capable of reacting with either

anti-A or anti-B has been isolated from *Ascaris* (154) and other helminths (155).

Work on the new Rh blood factors has progressed at a very rapid rate. Several reviews are available (19, 210, 211).

### ANTIBODY-ANTIGEN REACTIONS

*Optimum proportions.*—The determination and meaning of the "optimum proportions point," which finds practical application in the assay of antitoxins, for example, has been discussed by a number of authors (18, 120). Several attempts to account for the difference between the  $\alpha$  and the  $\beta$  optima have been made (12, 24, 28, 200). These are based chiefly on the assumption that different classes of antibodies and antibody-antigen compounds have different solubilities. Boyd & Purnell (24) also point out that the  $\alpha$  and  $\beta$  optima can never really coincide, even theoretically, since the two procedures are essentially different.

*Mechanism of antibody-antigen reactions.*—Two principal theories, the Bordet (15) hypothesis and the alternation ("lattice," "framework," "mutual multivalence") hypothesis (89, 120, 158) have been under discussion for some time. They have been tested by various experiments. The Bordet hypothesis would predict mixed aggregates in mixed systems (antigen A, antibody anti-A, antigen B, and antibody anti-B). Mixed aggregates have sometimes been observed, especially with concentrated antibody, and the velocity of precipitation in mixed systems has sometimes been found to be increased (101), but, on the whole, when antibody is not present in excess, the aggregates tend to be homogeneous, which observation agrees with the prediction of the alternation theory (1).

Marrack pointed out that, if the alternation hypothesis were correct, haptens with only two combining groups (divalent) might precipitate with antibody. A number of experiments have been made to test Marrack's assumption (17, 91, 100, 102, 160). Some divalent haptens have failed to precipitate with antibody, but others have been observed to precipitate. However, since such haptens, like many, if not most, organic dyes, are mostly aggregated in aqueous solution, the test is not strictly a rigorous one (22), unless a hapten which can be shown to be molecularly dispersed under the conditions studied can be induced to precipitate. The aggregation, and resulting colloidal character, was thought

by Landsteiner & van der Scheer (116), who first observed the specific precipitation of simple synthetic substances by antibody, to be the most probable explanation. Other observations indicate a general sort of correlation between the degree of aggregation of a hapten and its precipitability by antibody (21). Also the solubility of the compounds has an important effect (17, 54). On the other hand, we must record the fact that no hapten containing only one combining group for antibody has yet been observed to precipitate. This may be considered, on the whole, as favorable to the alternation hypothesis.

Quantitative theories of the precipitin reaction have been developed by various workers on the basis of the alternation ("multi-valent") theory (6, 89, 96, 97, 111, 160). Teorell (200) has proposed a seemingly equally successful mathematical treatment based on the assumption that antibody behaves as if it were univalent.

### COMPLEMENT AND COMPLEMENT FIXATION

Recent advances in the chemistry of complement have been discussed by Pillemer (167). The terminology of Pillemer, Ecker & Heidelberger for the components of complement has now become pretty general. C'1 designates the "mid-piece"; C'2, the "end-piece"; C'3, the relatively heat stable third component; and C'4, the fourth component, which is also heat stable, but not absorbed by yeast.

At a temperature of 1° C., components C'1, C'2 and C'4 combine with sensitized sheep erythrocytes, while C'3 does not combine. C'1 will combine with sensitized cells in the absence of C'4, but is hemolytically inactive unless C'4 combines previously or simultaneously. C'4 does not combine in the absence of C'1 (168).

Although C'3 is not fixed by sheep cell-antibody complexes, it is nevertheless essential for hemolysis, and operates on the sensitized cell after C'1, C'2 and C'4 have combined. C'3 thus behaves like a catalyst.

The chemistry of human complement has been studied by Pillemer, Ecker and co-workers (50, 51, 55, 56, 57, 58, 167, 169, 187). Under the proper conditions and with the proper concentrations, all the corresponding components of human and guinea pig complement are mutually substitutive (56). The relationships of



the complements of different species have recently been discussed by Cushing (40).

Haurowitz & Yenson (86) computed that  $1.5 \times 10^{-14}$  gm. of complement is required to hemolyze a single erythrocyte.

The anticomplementary properties of human  $\gamma$  globulin and the inhibition of this action by other substances have been studied by Davis *et al.* (41). The anticomplementary action was decreased by heating to  $56^\circ$  for half an hour, and was abolished by the addition of approximately equal amounts of serum albumin or  $\beta$  globulin.

### PRACTICAL USE OF ARTIFICIAL IMMUNITY

Adequately controlled experiments made during the recent war on vaccination against malaria indicated that this procedure, as carried out, was unsuccessful (88).

Methods of preparing and preserving killed typhoid vaccines, still retaining considerable Vi antigenic activity have been reported (3, 174) since the original work of Felix.

The use of antigens chemically separated from microorganisms has proven successful in a number of cases, sometimes after chemical treatment designed to render such antigens either less toxic or more potent. Heidelberger (87) was able to immunize to a number of pneumococcus types (using specific polysaccharides) so as to break up epidemics in the armed forces of the U.S.A. during the late war. More recent work with B.C.G. and other living avirulent vaccines in this country has suggested that they may be of definite value (52, 172, 197).

Goebel *et al.* (80, 164) reported that inoculation with the purified specific antigen of type V *Shigella paradysenteriae* (Flexner) produced agglutinins and mouse-protective antibodies in man. Morgan & Schütze (149) found that a species antigen from pneumococci, carefully separated from an "opposition factor," would immunize mice. Dubos (53) has pointed out that in general it is the surface antigens which are needed for effective immunization, but that not all surface antigens will work. Treffers (204) was able to detoxify soluble antigens from *Shigella dysenteriae* (Shiga) and *E. typhosa*. Evans (64) used formaldehyde to detoxify a toxic extract of *H. pertussis*, obtaining an antigenic product. Smolens & Mudd (193) isolated a portion of *H. pertussis* which was thought to

be useful in skin tests for susceptibility to whooping cough and possibly of some value in reinforcing waning immunity. Even a synthetic antigen, if it possessed the proper specificity, should produce the desired immunity, and an approach to this has been made (77 to 79). Good immunization seems to be obtained from the injection of alum-precipitated gas-gangrene (*Perfringens*) toxoids (118).

Mueller *et al.* (152) have shown that satisfactory antitoxin response is obtained by the injection of tetanus toxoid made from a toxin from bacteria grown on a purely synthetic medium. This toxoid gives fewer undesirable reactions than the older preparations, and the routine use of it or some similar preparation seems becoming widespread.

Toomey (202) has discussed the production of active immunity to smallpox, diphtheria, whooping cough, tetanus, and typhoid in an excellent review article.

Formalin-killed influenza virus seems to have proved effective as a vaccinating agent (94, 98, 196), although the duration of immunity may be limited, and seems to be restricted to the strain of virus used, as it is following recovery from the actual disease. Burnet & Clark (32) have presented a general discussion of immunity to influenza.

As by-products of the fractionation of human plasma, which was carried out on a large scale during the war to produce human albumin for use as blood substitute, globulin concentrates rich in various antibodies usually present (presumably as a result of previous attack or exposure) in human serum have become available (38). A  $\gamma$  globulin concentrate has proven successful in the prophylaxis and treatment of measles (105, 156). Preliminary studies indicate some value in the prevention and therapy of scarlet fever (105).

### DETOXIFICATION

The body's ability to detoxify poisonous substances is another important part of the defense mechanism. We can cover the recent literature only briefly here.

*Antimony.*—Goodwin & Page (81) recovered trivalent antimony from the urine of humans who were injected intramuscularly with pentavalent antimony. With emulsions of mouse liver, 9 per

cent of included pentavalent antimony or sodium antimony gluconate was reduced to trivalent antimony within twenty-four hours. Comparatively, the reduction of pentavalent antimony by living tissue was less rapid than the reduction of pentavalent arsenic.

*Arsenic.*—Astrachan (7), who has used liver extracts in arsenic therapy with beneficial effects, declared that the chief importance of liver lay in its detoxifying ability. Protection against the lethal effects of neoarsphenamine was afforded by *p*-aminobenzoic acid without interfering with the trypanocidal activity. Reduction of pentavalent arsenicals to trivalent arsenoxides was followed *in vivo*. Sandground (181) indicated that a high degree of structural similarity between toxicant and detoxicant was not an essential mechanism underlying detoxification phenomenon.

Martin & Thompson (122) found that ascorbic acid and cysteine were effective agents in the detoxification of the trivalent arsenical arsphenamine. The primary function of the ascorbic acid was to prevent oxidation after injection.

#### AROMATIC COMPOUNDS

*Sulfa drugs.*—Some of the newer sulfa drugs excreted as the acylated derivative are sulfadiazine (75), sulfathiazole and sulfathiazoline (68), 2,4'-aminobenzene sulfonylamino-4,6-dimethyl pyrimidine (sulfamethazine) (76, 175), and sulfamerzine (180, 207), although the latter was eliminated more slowly than most related drugs. The free compound was usually found (74). The monohydroxy derivative of sulfapyridine was found both free and as the glucuronide by Weber, Lulich & Major (208), Scudi (183), and Scudi & Jelineck (184). Sulfanilylbenzamide was excreted free and conjugated. Bose & Ghosh (16) found that the total excretion was 79 per cent in seventy-two hours, of which 58 per cent was free and 42 per cent conjugated. This proportion was reversed with sulfanilamide. The liberation of a free sulfonamide, which was presumed to be sulfathiazole, was found by Shay, Komarov, Siplet & Fels (190) after administered *N*⁴-phthalylsulfathiazole was removed by liver cells and excreted in the bile.

Karel & Chapman (109) concluded that vitamin C had no influence on the acute toxicity of sulfanilamide for guinea pigs.

*Hydrocarbons and halogen derivatives.*—An increase of urinary

hippuric acid following exposure to toluene in humans was found by Carpenter, Shaffer, Weil & Smyth (34). Styrene likewise gave an increased excretion of hippuric acid in humans.

*Nitro compounds.*—Von Oettingen and co-workers (45, 117) indicated that ascorbic acid fed to dogs and guinea pigs had no effect on the toxic manifestations of trinitrotoluene poisoning.

*Phenols.*—Deichmann (44) found that, in rabbits and rats treated with phenol, the bulk of urine phenol was present in a conjugated form. The phenol was removed from the body by excretion, oxidation, and conjugation, the latter two processes starting before the symptoms of poisoning appeared. The bulk of the phenol was oxidized to carbon dioxide and water. Small amounts were converted to pyrocatechol and hydroquinone, and were excreted as such or conjugated in the urine. The conjugated forms hydrolyzed in the presence of air and were converted to colored substances. Zondek & Shapiro (216) noted an increase in the amount of glucuronic acid and ethereal sulfates excreted following administration of *p*-chloro-*m*-xylenol to human subjects. Aerobic incubation of phenol with guinea pig liver slices produced about 20 to 30 per cent of the sulfate ester in the experiments of Bernheim & Bernheim (10).

De Meio & Arnolt (123) found by means of the Warburg technique that phenol conjugation by rat, guinea pig, puppy, and cat livers was inhibited by 0.002 *M* monoiodoacetate. This could not be re-established by the action of glucuronate. Inhibition was also produced by azide, octyl alcohol, fluoride, bisulfite, and partly by sodium oxalate. The lack of magnesium and the presence of citrate had no effect.

Rabbits with severe hepatic injury caused by a mixture of chloroform and carbon tetrachloride showed a significant impairment of the rate of phenol excretion as the organic sulfate.

*Amino and azo compounds.*—Egg white protein was found to have an ameliorating effect on the toxicity of azobenzene, *p*-amino azobenzene, and *p*-dimethylaminoazobenzene by Smith, Lillie & Stohlman (192). The urine of rats fed azobenzene was reported by Elson & Warren (62) to contain aniline and a water soluble derivative which, on treatment, yielded benzidine.

A large number of amino and azo compounds were fed rats by Miller, Miller & Baumann (145) and Miller & Baumann (143). These authors noted that each azo dye underwent a step-wise

demethylation *in vivo* prior to cleavage at the azo link. These demethylation reactions possibly occurred in the liver. Sodium acetate may serve as a detoxifying material, through an enzyme system, in poisonings with aromatic amines.

*Alkaloids.*—Anderson, Cornatzer & Andrews (4) reported that the removal of from one-third to one-half of the liver of rats given quinine caused an increase in urinary quinine from 72 to 143 per cent over that of intact animals. Andrews & Cornatzer (5) noted that about twice as great a percentage was excreted on an acid regime as on an alkaline one. The rest of the elimination of quinine and atabrine was believed by Chen & Geiling (37) to be in the capillaries, principally those of the lung. Seventy per cent of injected quinine was destroyed in five minutes in heart-lung preparations. There was no atabrine destruction for nearly one hour.

*Miscellaneous.*—It has been reported that some part of the unaccounted-for 40 per cent of penicillin is normally excreted from the animal body and is conjugated with glucuronic acid. Perlstein *et al.* (165) believed that, since commercial penicillin contains certain impurities, it was possible that the increased glucuronic acid in the urine may have been due to these impurities rather than to the penicillin itself. These authors injected penicillin into rabbits, and after daily determinations of the urine glucuronic acid and organic sulfate, they found that the latter did not change but that the glucuronate increased.

#### STEROLS

The intermediate metabolism of the sex hormones has been covered in an extensive review by Pincus & Pearlman (171) in 1943. In the same year a review of the literature on the steroid conjugates appeared by Venning (206).

Vitamin B deficiency was not found by Biskind & Biskind (11) to impair significantly the inactivation of testosterone propionate in the liver of male rats, although the inactivation of estrogens was impaired. Estrogens in the active and combined form were found in the urine of males with cirrhosis. Free androgens were not found. Endogenous androgens were inactivated after passage through the portal system and the liver (113), because prostatic implants were not maintained. Transplanted testes also failed to maintain prostatic tissue for a like reason.

The presence of  $\Delta^5$ -androsterone-3, 16-17-triol was reported to

be found in normal urine and in the urine of a boy with an adenocarcinoma of the adrenal cortex. This indicated to Marrian & Butler (121) that this compound was a normal steroid metabolic product.

Hepatectomy sensitizes the organism to the anesthetic action of steroids. In most cases, ablation of three quarters of the liver tissue increased the sensitivity of the organism four or five times, according to Selye & Stone (188) who worked with testosterone and methyl testosterone. Different steroids were not equally well detoxified by the liver.

Antifibromatogenic substances such as progesterone and desoxycorticosterone acetate was found by Dosne (46) and Eversole & Gaunt (65) to be somewhat inactivated by liver. Natural adrenal cortical secretions of the rat were not inactivated by the liver.

Zondek, Sulman & Sklow (217) reported that stilbestrol was inactivated by liver preparations less rapidly than estrone.

The fate of the estrogens has been the subject of much discussion. Heller & Heller (93) reported that  $\alpha$ -estradiol was inactivated by liver, partially by kidney, but not by the spleen or the heart. Estrone was inactivated by liver and kidney. Following a single injection of  $\alpha$ -estradiol, Schiller & Pincus (182) recovered 1 per cent of the estradiol as well as 217 per cent estrone and 5.4 per cent estriol. They decided that estriol was not converted to estrone or estradiol *in vivo*. Estrone and  $\alpha$ -estradiol were intraconvertible; injections of both gave urinary estriol. According to Pearlman & Pincus (161), men are able to convert administered estrone to estradiol. The liver is regarded by Segaloff (185) as the site of extensive inactivation of estrogen.

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DEPARTMENT OF BIOCHEMISTRY  
BOSTON UNIVERSITY SCHOOL OF MEDICINE  
BOSTON, MASSACHUSETTS  
ROCKEFELLER INSTITUTE  
PRINCETON, NEW JERSEY

## PHARMACOLOGY

BY C. C. PFEIFFER AND E. R. LOEW

*Department of Pharmacology, University of Illinois College of Medicine,  
Chicago, Illinois*

The recent release of the four-year accumulated results of the numerous pharmacological research studies allied to the war effort presents, due to immense bulk alone, a perplexing problem to a reviewer. The only, and admittedly inadequate, solution to this war-surplus problem is to omit those phases of pharmacology which, because of their immense popularity, are reviewed almost monthly by press and commercial news releases. As a stopgap to the time when all pharmacological contributions can be reviewed yearly in an urgently needed volume, the authors will review certain interesting phases of war research, and will also attempt to summarize certain important new developments which have resulted from that very small fraction of civilian research which literally fought for its first few respiratory gasps during the war period. Thus, data on anesthetics, anticonvulsants, analgesics, hypnotics, sympathicolitics, knowledge gained from radioactive isotopes, biometrics, hormone therapy, burn therapy, insect repellants, insecticides, antibiotics, antimalarials, parasitocides, sulfonamide chemotherapy, and toxicology, together with many lesser but no less meritorious advances in pharmacology will have to be omitted. Certain short reviews have been introduced to stimulate perhaps greater effort in chemotherapeutic fields where the need is great.

### PHARMACODYNAMICS

*Antispasmodics.*—Recent reviews (1, 2) discuss antispasmodics from the standpoint of chemical nature, methods of evaluation and pharmacological properties. Therefore, references herein are usually restricted to those dealing with progress made since 1943 in the development of antispasmodic drugs exclusive of the sympathicomimetic amines.

Alkamine esters of di- and tri-substituted acetic acid which have been studied extensively include esters of diphenylacetic acid (3, 4, 5, 6, 7, 8), benzilic or diphenylhydroxyacetic acid (3, 4, 9, 10, 11), and of substituted  $\alpha$ -thienylacetic acid (12, 13, 14). Compounds with anticholinergic activity approaching or equalling that

of atropine are obtained when acetates are trisubstituted, wherein an hydroxyl group is combined with  $\alpha$ -thienyl, phenyl or cyclohexyl groups (9, 12, 13), and greatest activity may obtain in quaternary alkamine esters. Several quaternary benzilic alkamine esters are more active than atropine with respect to mydriasis, antisalivary action and effect on blood pressure (9, 11). Benzilyloxyethyl dimethylethylammonium chloride (Lachesine) is a short acting mydriatic and cycloplegic (9, 11). Following injection, the quaternary derivatives of atropine, *l*-hyoscyamine, eucatropine and several benzilic esters (9, 11, 15) are more effective than the parent drugs with respect to the effects on blood pressure in the cat, salivary secretion in the cat and man (16), and the effect on pupil size in the mouse, but such differences may not be apparent after local application to the eye.

Benzilic esters of quaternary alkamines analogous to choline, and the benzilic ester of choline itself, antagonize acetylcholine and are truly atropine-like (9, 11). Since choline esters and ethers are well known for their cholinergic (muscarinic), nicotinic and curare-like actions it is important to consider the finding that an atropine-like, instead of cholinergic action, is possessed by benzilic esters of choline (9), by benzyhydriyl ethers of choline (53, 56, 57) which are also potent antihistamine agents, and by the dibutylcarbamic ester of choline (Dibutoline) which possesses useful mydriatic and cycloplegic activity (cf. 18 for refs.). With each of these types of compounds the greatest atropine-like action is exerted by those compounds in which one methyl group of the choline ester or ether is replaced by an ethyl group (11, 57).

Those esters of benzilic acid (9, 11) and substituted  $\alpha$ -thienyl-acetic acid (12, 13, 14) which possess greatest antispasmodic action are also most effective in producing mydriasis, inhibiting salivation and in blocking the depressor action of acetylcholine. A number of these compounds exhibit some degree of selectivity for certain tissues or organs and may prove to be useful mydriatics or secretory inhibitors. Compounds possessing activity comparable to atropine should have a high degree of selective action in order to obviate undesirable side-actions if they are to be used as uterine or intestinal antispasmodics.

Effective antispasmodics with a lesser degree of anticholinergic action than the compounds already mentioned include diphenyleneacetic (fluorene-9-carboxylic) acid ester of diethylaminoethanol (Pavatrine) (3, 19, 20), and alkamine esters of dihydroanthracene



and xanthene (17, 20). From a series of benzofuranone derivatives, 3-( $\beta$ -diethyl-aminoethyl)-3-phenyl-2-benzofuranone  $\cdot$  HCl (Ame-thone) was selected as a promising antispasmodic (21). Lobelan and related piperidine compounds possess analeptic and antispasmodic properties (22). It will be interesting to learn whether the antihistamine action exerted by some of these compounds on intestinal, uterine and bronchial muscle constitutes an improvement in the papaverine-like properties and whether clinical utility has been enhanced. It is important to note that the antispasmodics which relax uterine tissue of both the rabbit and human are those which possess powerful antihistamine and antiacetylcholine action (23).

*Antihistamine drugs.*—The recent availability of potent, non-toxic antihistamine agents is of major significance since they can be used as research tools as well as for therapeutic and diagnostic agents. Literature reviews (29, 30, 31) cite convincing evidence in support of the thesis that histamine causes the major symptoms of anaphylaxis and allergy. This evidence, in conjunction with the recent demonstrations that antihistamine drugs alleviate symptoms of anaphylaxis and are therapeutically effective in allergic diseases (for refs. cf. 32, 33, 34, 35), constitutes proof that histamine plays a prominent etiological role in anaphylaxis and allergy. Antihistamine drugs should prove to be valuable tools for use in determining whether histamine is involved in a variety of physiological and pathological conditions (36).

Compounds with some degree of antihistamine specificity were discovered by Staub & Bovet (37) and Staub (38) when studying phenolic ethers and phenylethylenediamines. The ability of 2-isopropyl-5-methylphenoxyethylamine (929F) and N-phenyl-N-ethyl-N'-diethylethylenediamine (1571F) to alleviate anaphylactic shock and histamine-induced bronchoconstriction and to prevent histamine from contracting intestinal muscle has been confirmed (cf. 32, 39, 46, 80 for refs.). Further studies revealed that 929F was a weak or ineffective antispasmodic (40) and analgesia has been demonstrated (41, 54) with doses which were undoubtedly toxic (40, 41, 54, 55). These compounds were too toxic for use in human beings. The dimethyl derivative of 1571F, designated as 2325RP, has been compared (42) with a more potent derivative, N-dimethylaminoethyl-N-benzyl-aniline or Antergan (2339 RP), which has since been used effectively in allergic diseases (32, 33).

In 1944, Bovet and his collaborators (43, 44, 45) first revealed

the remarkable antihistamine properties and potency of *N*-*p*-methoxybenzyl-*N*-dimethylaminoethyl  $\alpha$ -aminopyridine  $\cdot$   $H_3PO_4$  (2786 RP or Neoantergan). A homologue, Pyribenzamine or 63-C, which lacks the methoxy group contained in Neoantergan has similar properties (49 to 52, 81).

Discovery of antihistamine action in an extensive series of benzhydryl alkamine ethers led to the selection of  $\beta$ -dimethylaminoethyl benzhydryl ether.HCl (Benadryl) since it possessed a high activity in antagonizing histamine and an appreciable degree of antihistamine specificity (46, 48, 53). Quaternary ammonium derivatives of Benadryl are choline ethers which are unique by virtue of possessing anticholinergic as well as antihistamine properties (53, 56, 57).

Benadryl, and the two  $\alpha$ -aminopyridine derivatives, Neoantergan and Pyribenzamine, are remarkably effective in alleviating bronchospasm due to administration of histamine or its liberation during anaphylaxis in guinea pigs (44, 45, 46, 47, 48, 49, 50, 51, 52, 81). These agents prevent histamine-induced spasm of intestinal muscle of the dog (51, 58) and guinea pig (45, 49, 50, 53) and diminish the hypotensive action of histamine administered to dogs or liberated during anaphylaxis (28, 43, 51, 59, 60, 61). They also diminish the increased capillary permeability demonstrable in normal rabbits following intradermal injections of histamine (62, 82). Benadryl and Neoantergan fail to alter the changes in capillary permeability induced with various other agents (trypsin, venoms, codeine, tetracaine) which were claimed or suspected of causing liberation of histamine from the tissues (62 and cf. refs.). As additional evidence that histamine probably does not play an important role in pharmacological responses to trypsin is the fact that antihistamine drugs neither prevent trypsin from increasing capillary permeability in rabbit's skin (62) nor prevent the lethal effect of trypsin in guinea pigs and dogs (61).

In human beings, dermatographism and cutaneous reactions which follow intradermal injections of histamine, allergens, and snake venom are reduced after oral and topical administration of antihistamine drugs (63 to 68, 81, 83); reactions to ultraviolet irradiation are not diminished by Antergan (69). It is doubtful whether antihistamine drugs exert a specific inhibiting influence on the gastric secretagogue action of histamine (34, 53, 55, 70, 71, 72, 73, 74).

Benadryl differs from Neoantergan and Pyribenzamine in that it exerts an antispasmodic action (51, 53, 57) whereas the  $\alpha$ -aminopyridines will induce contraction of intestinal and uterine muscle in dogs (51, 58) and isolated guinea pig ileum (45). However, each drug antagonizes the spasmogenic action of histamine. Neoantergan and Pyribenzamine appear to be very potent with respect to preventing histamine-induced bronchoconstriction in guinea pigs but with respect to alleviation of anaphylaxis and diminution of the hypotensive action of histamine, Benadryl and the  $\alpha$ -aminopyridines exhibit potencies which are equal or not as widely divergent (51, 52, 62). Augmentation of the pressor action of epinephrine is not due to an atropine-like action (51). Several antihistamine drugs produce sedation in man (34, 35, 72). The phenomenon is unpredictable, it may disappear after continued dosage, and will have to be studied in human subjects since in several animal species toxic doses induce tremors and convulsions and not depression or hypnosis. No evidence of chronic toxicity was encountered when Benadryl was administered orally to dogs over a period of several months (75) or when Pyribenzamine was incorporated in the diet of rats (76); their acute toxicity in mice is similar to that of Neoantergan (45, 51, 75, 76), the LD50 ranging from 75 to 90 mg. kg., intraperitoneally.

Antihistamine drugs, unlike epinephrine and related amines, do not act by producing pharmacological responses diametrically opposed to those induced by histamine. Effective doses may not elicit any pharmacological reaction, yet are capable of blocking many of the diverse actions of histamine, apparently by competing with histamine for absorption on to the effector mechanism (59, 80).

In addition to epinephrine and atropine the nonspecific antispasmodic drugs which have some action in alleviating anaphylaxis and histamine shock in intact guinea pigs and antagonizing the spasmogenic action of histamine on intestinal muscle are theophylline (48, 77, 78), papaverine (38, 47, 79),  $\beta$ -diethylaminoethyl phenylpropylacetate (Propivane) (42), isonipecaine (Demerol) (47), esters of 9,10-dihydroanthracene (17), several alkyloxytriazines (78), and 3-( $\beta$ -diethylaminoethyl)-3-phenyl-2-benzofuranone-HCl (Amethone) (23). The amino acids, arginine, histidine and cysteine, as well as derivatives of arginine and histamine which lack a free amino group, are nonspecific histamine antagonists of low potency (24, 25, 26, 27).

## CHEMOTHERAPY

**Fungicides.**—As a direct result of the military needs of tropical warfare many new mildew-proofing and fungicidal agents were developed. Some of these are copper naphthenate, copper pentochlorophenate (84), zinc dimethyldithiocarbamate, phenyl mercurioleate, pyridyl mercuristearate, dihydroxy dichloro diphenyl methane, salicylanilide, octadecyl trimethyl ammonium pentochlorophenate (85), and pentochlorophenate. The only immediate pharmacological interest of these fungicides is the fact that most of them will produce contact dermatitis and sensitization of the skin of those workers who handle the treated products.

Of greater pharmacological interest are the naturally occurring fungicidal fatty acids which seem to have been introduced to therapeutics by a study of the fungicidal agents in sweat (86, 87), and the discovery that sodium or calcium propionate is an effective dusting powder to prevent the growth of molds on bread ("mycoban," DuPont). Numerous workers (88, 89) have confirmed the *in vitro* fungicidal and fungistatic action of both propionic and undecylenic acids. The fungistatic concentration against *Tricophyton gypseum* is as follows: propionate 1.0 per cent, valerate 1.0 per cent, caporate 1.0 per cent, caprylate 0.1 per cent, caprate 0.1 per cent, undecylenate 0.1 per cent, benzoic 5 per cent, and Lugol's solution 10 per cent. The activity of fatty acids increases with chain length within solubility limits (89). This *in vitro* effectiveness has been substantiated by the clinical results where in prophylactic tests a dusting powder consisting of 2 per cent undecylenic acid, 20 per cent zinc undecylenate, and 78 per cent talc allowed an incidence of 1 per cent; standard boric acid—salicylic powder 11 per cent; and 20 per cent sodium propionate powder 3 per cent; and controls 9 per cent active fungous infections of the feet (90). Groin infections were decreased from 10.3 to 0.7 per cent by the use of the powder prophylactically. The complete lack of irritation or sensitization of the skin recommends this type of treatment although the treatment must frequently be continued indefinitely to prevent a recurrence (91, 92, 93). Ten per cent sodium caprylate has been recently recommended as being superior to the other fatty acid ointments (94, 95) and the fatty acids in general have been found to be moderately active bacteriostatics (96). The high incidence of pre-pubertal infections of the scalp by *Microsporum audouinii* (ringworm) when compared to the relative immunity of

adults now has a logical explanation in the finding that adult hair contains a fungicidal fatty acid which is fungistatic for *M. audouini* in concentrations of 0.0002 to 0.001 per cent (97). The chemical analysis of extracts of the fat on adult human hair reveals the natural fatty acid to be C<sub>7</sub> to C<sub>11</sub> in chain length and more recent data indicates the fatty acid to be C<sub>9</sub> (saturated). Presumably the increased fat gland secretion which accompanies puberty provides this fungistatic secretion. The antibiotics clavacin, streptothricin (98), and viridin (99) may also gain places as effective fungicidal agents but only meager data are as yet available.

*Antimony.*—The specific chemotherapeutic effect of tartar emetic and other antimonials in kala-azar (which is ordinarily fatal) is reenforced by the report on the antimony treatment of 585 cases of visceral leishmaniasis with only 5.1 per cent mortality (100). Pneumonia was the most common complication of the treatment. Antimony treatment of filariasis in America has perforce been limited to animal infections since few if any of the men in the American armed forces developed circulating microfilaria and the initial lymphangitis was self-limited (101). However, numerous authoritative clinical papers describing the use of antimony derivatives were published as follows: in filariasis-anthiomaline (102) and neostibosan (103); in schistosomiasis-fuadin, tartar emetic, anthiomaline (104), anthiomaline, stibophen (105) and urea stibamine (106); in leishmaniasis-neostam, neostibosan (100), aminophenylstibinate of methyl-glucamine (107), neostibosan (108) and the non-antimonial 4:4'diamidinostilbene (109) which was apparently effective in five cases which were presumed to be antimony-fast. Anthiomaline was effective in lymphogranuloma venereum (110) and Diramin (111) was effective in granuloma inguinale. The description of a case of induced *Schistosoma hematobium* infection in a volunteer (112) provides excellent teaching material as does also a case history with autopsy findings (113).

Several suitable animal hosts have been found to serve as test animals for the screening of new antimonial compounds. In trypanosomiasis of the mouse, stibamine was the most effective and tartar emetic had the least activity of eight compounds (114). The acid salt of the antimony analogue of "melarsen" is relatively insoluble and produces a prolonged chemotherapeutic and prophylactic effect in mouse trypanosomiasis (115,116). Neostam (117) and stibamine glucoside (118) were highly effective in filariasis of

cotton rats, as is also the well-tolerated pentavalent drug Stibanose (119). In a series of 185 compounds, of which 30 were antimonials (120), none was found to exceed stibanose, neostam, or neostibosan in the treatment of leishmaniasis of the hamster which, if untreated produces extensive amyloidosis in this host (121). Intravenous toxicity studies wherein LD50's are expressed in antimony content indicate a wide variation in toxicity and greater toxicity of the trivalent compounds. The toxicity of urea stibamine depends in part upon the antimonius acid content (122).

Excretion and distribution studies of antimony made with the polarograph, the rhodamine-B method, and the use of radioactive antimony are in fair agreement. The strong iron line at wave length 2598A interferes in the attempted spectroscopic determination. On polarographic analysis, mice excrete 30 and 35 per cent of intraperitoneally injected tartar emetic within twenty-four and forty-eight hours respectively. After intravenous dosage the excretion in twenty-four and forty eight hours was 62 and 68 per cent respectively (123). Using tartar emetic and isotope technique in heart-worm infestations of the dog, antimony injected intravenously was found to leave the blood stream in a matter of minutes and localize in the liver (10.7 mg. per kg.), thyroid (3.8 mg. per kg.), and adult filarid (1.5 mg. per kg.) (124). With repeated injections over a twelve day period antimony accumulated in the thyroid (18 mg. per kg.) which exceeded the liver (14 mg. per kg.). To the despair of those who advocate exact plasma levels to determine all chemotherapeutic effects, the blood antimony, even after continued injection, is all in the red cells. A similar tissue distribution of radioactive antimony (injected as tartar emetic. 0.8 mg. per kg.) was found in the hamster infected with *Schistosoma mansoni*. The wet tissue levels one hour after injection were: liver 4.5, parasites 4.0, and thyroid 2.0 mg. per kg.; at four hours, liver 14, thyroid 4.0, spleen 3.0, and parasites 2.0 mg. per kg.; and at twenty-four hours, liver 9.0, parasites 2.0, kidney 1.0, thyroid 0.5, and spleen 0.3 mg. per kg. (125). After intravenous dosage in the hamster the trivalent antimonials, tartar emetic and anthiomaline, are stored to the extent of 15 per cent in the liver while the quinquevalent compounds are only present to 5 per cent of the injected dose, but a greater amount is present in the spleen. With trivalent compounds urinary excretion accounts for approximately 15 per cent, while fecal excretion accounts for 50 per cent of the excreted

antimony. The corresponding findings for the quinquivalent compounds are 70 per cent urinary and only 4 per cent fecal excretion (126).

*Rickettsiostatic agents.*—The discovery of a chemotherapeutic agent effective in the treatment of rickettsial infections represents a real advance since this organism is an obligate intracellular parasite, a class which like the viruses has been hitherto resistant to therapy. One of the first drugs found to be effective by German workers in murine typhus infections of mice and guinea pigs was methylene blue (127, 128). Following the use of toluidine blue and Forbisen (129) for experimental murine typhus infections it was independently found that methylene blue (130) was more effective as a rickettsiostatic agent than toluidine blue or *p*-amino benzoic acid (PABA) (131) in scrub typhus infections of the mouse. Chronic toxicity studies (132) proved methylene blue to be extremely toxic to the higher mammalian erythrocytes with fatal anemia sometimes resulting after five days of therapy. This red blood-cell-destroying effect of methylene blue was confirmed by human studies in the therapy of scrub typhus.

As a chemotherapeutic agent *p*-aminobenzoic acid (PABA) (131) is much more encouraging and has already proven its worth in many clinical types of rickettsial infections. The chemotherapeutic effect in murine typhus was independently confirmed (133). The first clinical trial was in louse-borne typhus (134) where initial doses of 6 to 8 gm. PABA, followed by 2 gm. every two hours, decreased both the severity of the disease and the fatality rate. Following the finding that the mortality rate of experimental scrub typhus (135, 136) could be reduced with the oral or parenteral administration of PABA, the therapy was then carefully tried in patients with scrub typhus (137) in Army hospitals in India. On a regime of 8 gm. initial dose and 3 gm. every two hours, the patients developed blood levels of 30 to 60 mg. per cent PABA within two days. When alternate cases were treated with the drug the beneficial effects noted were marked reduction of fever within two to five days, and disappearance of lymph adenopathy, headache, and toxemia. No deaths occurred in the treated group, while three out of sixteen control cases died. When therapy was stopped too soon, the fever and symptoms returned. No serious toxic symptoms occurred in any of the treated patients except mild delirium in those patients with blood levels above 100 mg. per cent. Two



patients developed neutropenia with white counts below 3,000 per c. mm. but therapy did not have to be interrupted. One-half gm. of sodium bicarbonate was given for each gm. of PABA to lessen the gastric irritation. The chemotherapeutic effect is apparently less evident in endemic typhus (138). PABA has also been shown to be effective in both experimental spotted-fever infections of guinea pigs (139) (where 2 per cent dietary levels will prevent mortality), and in clinical spotted fever (140).

The mode of action of either PABA or methylene blue is not known but it is evident from the normal rise of agglutinin titre in the treated experimental animals and the fact that the tissues of the animals treated with either PABA or methylene blue still harbor the rickettsia in spite of large continued chemotherapeutic doses that only attenuation of the infection or a rickettsiostatic effect obtains (141). A careful and systematic study of PABA in rickettsial infected eggs (142) showed the molecule to be rigidly specific since the ortho and meta isomers of PABA, lengthening of the carbon chain so as to remove the carboxyl group from the ring, ring substitutions of any kind, and *p*-amino acetophenone were all completely inactive at the effective PABA dosage.

Various *p*-sulfonamido-benzamidines (143) were prepared and studied in England, and thirteen were found to be somewhat effective in murine typhus infected mice. The reports on clinical trial of these compounds is not yet available. Dimethyl phthallate (144) or dibutyl phthallate (145) may be used on clothing as effective mite repellants to prevent scrub typhus.

*Viricides.*—That shelf which contains our armamentarium of agents effective against virus infections is still bare. The negative results obtained after testing 190 compounds in the two neurotropic viruses, poliomyelitis and encephalitis, are reviewed (146) together with a chart summarizing the effects of all compounds that have ever been tried in virus infections. The beneficial prophylactic effect of atropine in intranasal influenza inoculations of mice is reported (147). Neither Prontosil nor Neoprontosil modifies the course of lymphocytic choriomeningitis virus infections in the guinea pig or mouse (148). Neoarsphenamine and tryparsamide are somewhat more effective than mapharsen in experimental murine poliomyelitis (149). Thiouracil slightly shortened and thyroïd extract or thyroprotein significantly prolonged the incubation period of mice inoculated intracerebrally with poliomyelitis virus

(150). Ether anesthesia delays the onset of encephalitis symptoms in the mouse (151). Streptomycin and PABA were ineffective, while penicillin and sodium sulfadiazine had a marked inhibitory effect on the growth of psittacosis virus in both tissue cultures and eggs (152), but patients with psittacosis were not benefitted when treated with sulfapyridine or sulfathiazole (153, 154). A study and review of antibiotics found three active *in vitro* against fowl pox virus and one against laryngotracheitis virus (155). A series of twenty-four compounds which included sulfonamides, acridines, antibiotics and an arsenical were inactive in influenzal virus infections of mice (156). Histamine may serve as a possible aid in establishing various infections in new hosts since fatal intravenous herpes simplex virus infections may be produced in histamine pre-treated rabbits (157).

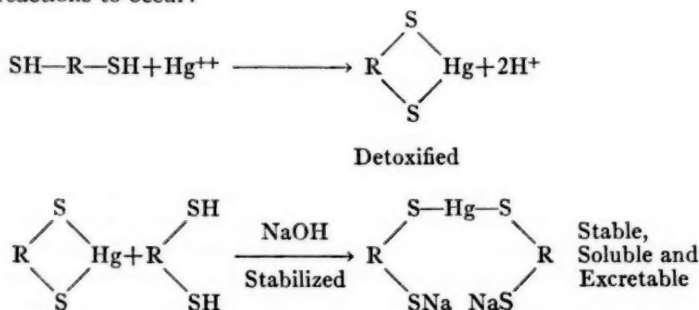
*Chemotherapy of cancer.*—As a culmination of extensive studies of the chemotherapy of mouse leukemia, the British have observed a definite chemotherapeutic effect of urethane in leukemic patients (158, 159). The response in lymphatic leukemia is less satisfactory than that in myeloid leukemia. A major toxic reaction may be a general depression of the bone marrow with resultant aplastic anemia although ordinarily with the decrease in abnormal white cells the red cells usually increase. A minor toxic symptom, namely nausea, was the limiting factor in dosage. Weekly anesthetizing doses of urethane will increase the incidence of lung tumors in C3H mice from 5 to 75 per cent (160), but phenyl urethane and isopropyl phenyl-carbamate cause a retardation of spontaneous mammary cancer in the mouse and also retard Walker rat carcinoma No. 256 (161). When tested against spontaneous mammary carcinoma of mice, 132 common chemotherapeutic chemicals were ineffective (162). The growth-inhibiting effect of bis and tris nitrogen mustards has been found beneficial in Hodgkins' disease, but the thrombocytopenic effect is too great to afford a safe treatment of leukemia (163). Of a series of cyclic nitrogenous compounds, several diaminodiphenyl derivatives showed promise (164), as did also a group of quaternary compounds (165) tested against mouse tumors. Another interesting approach is the use of a brominated triphenyl estrogen in the treatment of mouse tumors (166). Of a series of unsaturated dibasic acids, maleic and citraconic anhydrides greatly retarded skin tumors induced with 3,4 benzpyrene or 1,2,5,6 dibenzanthracene (167). Suramin (Germanin), and other

azo dyes when tested on transplanted lymphosarcomas of the mouse, produced some inhibition of growth but transplantability of the tumor was retained (168).

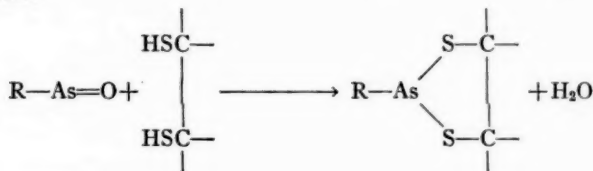
The ability of colchicine to arrest mitosis has led to its trial in animal tumors and human leukemia (169). Toxic doses are required to retard the growth of tumors and the effect of tolerated doses in man is only temporary. As predisposing factors to cancer the combination of low thiamine and high estrogenic level may be significant (170) and the tissue changes in mice after pentose nucleotides (adenylic, guanylic, cytidylic and uridylic acids) produce lesions suggestive of sarcoma (171). After a year's observation of eight cancer patients on a high avidin diet no definite curative effect was discernable (172). Coley's toxin may owe its anti-carcinogenic action to a specific factor which decreases the capillary blood supply of the tumor (173).

#### TOXICOLOGY

*Detoxication with Sulfhydryl Groups.*—Extending the pharmacological knowledge of the antagonism between sulfhydryl-containing compounds and heavy metals, the British found an important disulfhydryl-containing compound effective against Lewisite ("BAL" 2,3 dimercaptopropanol) (174, 175). This compound in ointment form has already saved the eyesight of many workers in poison gas shell loading plants and has reduced the mortality in bichloride of mercury poisoning (163). The most important advantage of this compound over cysteine hydrochloride or glutathione resides in the presence of two adjacent sulfhydryl groups on the same molecule which in the case of mercury allows the following reactions to occur:



A similar detoxication of an arsenoxide type of arsenical has been written:



At present BAL, "Dimercaprol" (176) is available for human use as a 10 per cent sterile solution in vehicle consisting of 20 per cent benzyl benzoate and 80 per cent peanut oil. Man will tolerate up to 5 mg. per kg. intramuscularly every three hours of BAL in this solution without severe toxic symptoms, whereas 8 mg. per kg. produces severe intoxication. Heavy metal poisoning should be treated diligently and continuously by an initial injection of 1/4 cc. per 10 lbs. body weight (4 cc. per 160 lb.) followed by 1/8 cc. per 10 lbs. body weight intramuscularly every three to four hours for several days.

In animals the generally tolerated, non-lethal intramuscular dose is approximately 100 mg. per kg. Doses in excess of this rapidly produce lacrimation, blepharospasm, conjunctival edema, salivation, vomiting, dyspnea, muscle tremors, rapid thready pulse, tonic or clonic convulsions, coma and death, which is probably due to the interference with enzyme metabolism in the body since BAL keeps cytochrome C in a reduced state. Glycolysis of CNS metabolites is also inhibited by the combination of BAL with the metal-protein complexes concerned in the enzymatic processes. For the simple demonstration of BAL detoxication of mapharsen or bichloride of mercury poisoning in animals it is desirable to use an initial dose of no more than 50 mg. per kg. followed by repeated smaller doses if necessary.

In reversing enzyme inhibitions produced by arsenic poisoning BAL exhibits a greater affinity for arsenic than do the tissue enzymes such as the SH groups in trypanosomes since if trypanosomes or sperm made immobile with arsenic are treated with adequate doses of BAL normal motility and morphology are promptly restored (163). This antagonism is even more dramatic *in vivo* in the infected rat where an intramuscular (50 mg. per kg.) dose of BAL given after a 5 mg. per kg. intravenous dose of mapharsen

will completely negate any chemotherapeutic effect of the arsenical on the trypanosoma equiperdum as shown by complete constancy of trypanosome counts during the experiment. This dose of mapharsen will ordinarily clear all of the trypanosomes from the blood stream within fifteen minutes. Moreover, BAL given thirty to forty-five minutes after the disappearance of *T. equiperdum* from the blood will still reverse the chemotherapeutic effect and allow the rat to die of trypanosomiasis (177). Thus the therapeutic effect of arsenicals is, completely nullified when sulfhydryl groups are used to counteract the toxic effect of arsenical chemotherapy.

Clinically the use of BAL has thus far been found effective in four conditions: (a) the toxic reactions sometimes resulting from the rapid treatment of syphilis (178); (b) bichloride of mercury poisoning; (c) cadmium fume fever; and (d) zinc fume fever. Experimentally BAL will also reverse vanadium, antimony, lead, and bismuth intoxication (175). "BAL-Intrav" (25 per cent) when sterilized by Seitz filtration is suitable for intravenous injection, and man tolerates doses as high as 4 grams (179). Copper excretion is increased to thirty times normal after "BAL-Intrav" is given intravenously in the sheep (180). In man, slow intravenous dosage (181) causes a twenty-fold increase in copper excretion and a five-fold increase in zinc excretion, but iron excretion is not altered. The sulfhydryl groups in the body are essential for the activity of many of the enzymes concerned with carbohydrate (182), fat and protein (183) metabolism.

Numerous other dithiols have been synthesized and tested (184) but the only compound which may be less toxic and more effective than BAL is the oxy-glucoside of dithioglycerol (185).

*Rodenticides.*—One of the most fascinating and practical research developments of the war period has been the discovery of two very potent rat poisons (186, 187, 188). Table I summarizes the objective and comparative findings.

The toxicity of the thioureas for the rat were first noted when a study on bitter blindness in rats was initiated (189). Following the usual method of testing taste ability in human beings, a few crystals of phenylthiourea were placed on the tongues of six rats and to the investigator's surprise all six animals were dead the next morning. This led to further tests on this and other specific poisons. Specificity of toxicity of alpha naphthylthiourea (ANTU) for the Norway rat is indicated by the following oral lethal dose for fifty per cent of the animals (LD50's) in mg. per kg. (190): Adult Nor-

way rat 6.5, dog 12, albino mouse 75, Alexandrine rat 25, rabbit 400, guinea pig 400, and monkey and chicken (LD100) 5000. As in methemoglobinemia, the herbivores are in general more resistant than the carnivores. Sodium fluoroacetate (1080) is a discovery of the chemical warfare service and is not nearly so specific in species toxicity as is shown by the following oral LD50's (mg. per kg.):

TABLE I  
COMPARATIVE EFFECTIVENESS OF NEW RODENTICIDES

Drug	mg./kg.*	Bait Level in Per- centage	Cause of Death	Comments
Sodium Fluoroacetate (1080)	5.0	0.5	Cardiac depressant and convulsant	Tasteless and odorless, useful against insects
Alpha Naphthyl Thiourea (ANTU)	6.5	0.5	Pulmonary edema	Mild emetic, mild taste, and odorless
Thallium Sulfate	16	2	Respiratory failure	Worker should wear rubber gloves
Strychnine	25	1	Convulsant	Kills mice but too bitter for rats
Zinc Phosphide	40	2	Fatty livers	Stabilized with calcium carbonate
Arsenic Trioxide	140	3	Enteritis and neuritis	Rats learn to avoid bait
Best Red Squill		10	Cardiac	Extremely
Females	150		Cardiac	variable
Males	300		Cardiac	potency
Barium Carbonate	1500	20	Cardiac	Emetic in all but rodents
Poor Red Squill	500+		Cardiac	

\* Oral toxic dose, Wild Norway Rats.

wild black rats 0.1, dogs 0.35 (convulsant poison), cats 0.35, meadow mice 0.5 goat 0.7, horse 1.0,<sup>1</sup> white rats 2.5, Norway rats 5.0, deer mice 5.0, and leghorn hens 10.0. Thus, 1080 is an all-purpose lethal agent, and may be used as a 0.5 per cent solution in drinking water against all rodents and even ants. In addition, 1080, occurs naturally in "Gifblaar" *Dichapetalum Lymosum* which is one of the most poisonous plants in South Africa (191). In contrast to birds the rat is color-blind so that unnatural green aniline dyes may be used in the baits to protect the bird. ANTU acts as an

<sup>1</sup> Not an LD50.

emetic in many animals, and tartar emetic may be used with 1080 to protect domestic animals other than rodents. All animals develop rapidly a tolerance to sublethal doses of ANTU which begins within three hours and lasts thirty days. For maximum kills, pre-baiting with unpoisoned baits is hence desirable. The extreme toxicity of these agents limits their use to the qualified professional exterminator (192, 193).

Young rats are five to seven times more resistant to ANTU (194, 195, 196). Both rodenticides are sufficiently toxic to be used as a dusting powder (20 per cent level) and the rats are killed by the poison licked from their feet. In the dog ANTU will produce an eighty-fold increase in the pulmonary lymph flow and adrenal cortical extract is partially antidotal. ANTU in doses of 10 mg. per kg. given intraperitoneally to the rat produces depletion of liver glycogen, rise in blood sugar, and prevents glycogenesis. One gm. per kg. of cysteine hydrochloride antidotes the lethality of 10 mg. per kg. of ANTU (197). When administered chronically ANTU prevents hair growth and normal pigmentation and has the characteristic effect of the thioureas on the thyroid gland. The cat develops jaundice as a result of intrahepatic obstruction (190). The dog made tolerant to ANTU develops a six-fold rise in serum cholesterol. Pretreatment of rats with increased dietary iodine or iodides increases the LD<sub>50</sub> from 6.5 to 46 mg. per kg. (198, 199). A tentative outline of the treatment for human poisoning with ANTU would consist of gavage, oxygen therapy with pressure breathing, and avoidance of fluids, particularly alkaline fluids. The administration of BAL or cysteine hydrochloride might also be of value. In studies with tissue slices and breis sodium fluoracetate inhibits specifically the oxidative metabolism of pyruvic and acetic acids probably by inhibition of dehydrogenation via the citric acid cycle (200). The only suggested antidote for 1080 poisoning is the intracardiac injection of procaine hydrochloride to prevent ventricular fibrillation and the symptomatic administration of barbiturates to control convulsions. Neither of these procedures has been very effective experimentally.

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